Mother-offspring vocal communication and temperament in cattle

Mónica Padilla de la Torre, BSc (Hons), MSc.
Universidad Nacional Autónoma de México

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

May 2013

Acknowledgements

I would like to thank my supervisors Dr. Tom Reader, Dr. Alan McElligott and Dr. Elodie Briefer. I have no words to express my appreciation to Alan, who gave me the opportunity to do this PhD in the University of Nottingham in first place. To Tom, for sharing with me his endless knowledge and love for science, for his patience and excellent sense of humour, but more than anything, for his constant academic and personal support. To Elodie, for her fundamental academic guidance in bioacoustics and for the great friendship we build up. This thesis would not have been accomplished without their support which I feel very lucky to have received.

Thanks to my examiners Dr. Francis Gilbert and Dr. David Reby for their feedback and suggestions which helped me to improve this research significantly.

I will be always grateful with the Hackett Family who had kindly received me in their farm and allowed me to study their cattle during three years. It was an extraordinary personal experience to have met with such kind family in that amazing environment. Endless thanks to Catherine, Desire and David Hackett.

Thanks to CONACyT for the studentship that funded this research and UFAW for funding the pilot study by Victoria Ratcliffe that lead to this research.

I would like to thank all the students that helped and collaborated with my fieldwork: Tom Dewey, Claire Farrington, Céline Charpin, and especially to Brad Ochocki, who gave relevant inputs during the last and more intense field work season.

I will be always thankful with Ertan Erol and Helena Ajuria Ibarra for becoming my family far from home. Thank you so much for all the emotional contention and for the deep and true friendship. I was lucky enough to have found extended family members; thanks to Tikva Meron, Kayvan Forouhesh, Elif Uzgören, Zeinep Özkurt, Ivette Fuentes and Leonardo Schuller...I would have never made it without your love and support.

I have no words to thank all the wonderful friends that gave me their support and friendship during my studies all these years, Brad Ochocki, Sehun Cheon, Laure Reis, Sarper Aydogan, Aycan Damali, Teresa Fritsch, Stine-Marie Simensen, Jaime Adame, Ed Tripp, Altair Contreras, Aliya El-nagar, Gürbüz Comak, Ali Kemal Karabulut... and also the support from good old friends back home, Verónica Custodio, Jimena Vergara, Sara González, Edith González, Christian Maciá, Monica A. García, Ireri Villamar, Hector Olguín, David Ortiz, Hector Bonilla.

Finally and very importantly, I would like to thank my family's unconditional love and support. Thanks to my mum Araceli de la Torre Siqueiros, always my best example of intelligence and courage, and my dad Miguel Angel Padilla Leyva for being there for me always. Many thanks to my tiny, but amazing family: Nadia Calderas, Roselia Padilla, Manuel de la Torre Siqueiros, Selene de la Torre and Manuel de la Torre and Silva González.

In memorian: To my Northern Star Eulalia Siqueiros and my Southern Star Aurora Leyva

Summary

Very little is known about cattle vocalizations. The few studies available in the literature have been conducted using animals under stress or very intensive husbandry conditions. Similarly, the individual consistency of behaviour in cattle has rarely been considered except in applied studies of constrained and isolated animals, and no previous research has attempted to address a possible association between vocal communication and temperament in cattle. The studies reported here address these gaps in our knowledge.

I found that cattle contact calls have acoustic characteristics that give them individualized distinctiveness, in both adult cows and calves. These results were confirmed using playback experiments, where I found that there is bidirectional mother-offspring recognition, as has been recorded in other "weak hider" ungulates. Additionally, using visual and acoustic stimuli, I assessed individual cattle temperament. The results showed that there was no individual behavioural consistency in responses to a novel object presentations. However, calves behaved consistently more boldly than cows. Furthermore, there was significant individual consistency in responses to vocalisations of heterospecifics, when they were played back through a speaker in the field. Surprisingly, no correlations were found between the ability of cattle to identify their own mother/offspring and the acoustic features of their vocalisations, or behavioural responses in any other context. There were, however, significant correlations between one characteristic of vocalisations in adult cows (formant spacing) and the boldness of

behavioural responses to both novel objects and auditory stimuli. Additionally, higher F0 in calf contact vocalizations correlated with boldness in the auditory stimuli experiment. These relationships imply that vocalisations may encode information about individual temperament, something which has rarely been documented. Surprisingly, no strong correlations were found between the behavioural responses to visual and acoustic stimuli, suggesting that individual consistency in behaviour across contexts was limited, and that behavioural plasticity could play an important role in determining responses in different environmental contexts. Overall, my results contribute to our knowledge of animal communication in mammals from a bioacoustic point of view, and they are also potentially relevant to studies of vocalizations as indicators of cattle welfare.

Table of contents

CHAPTER ONE

General Introduction

General introduction

1.1. Introduction

In order to provide a general overview of the natural history of the species studied in this thesis, as well as the topics that I will be discussing in more depth in the following experimental chapters, in this chapter I review the origins of modern domestic cattle, and the existing research into cattle behaviour. I end the chapter by identifying the objectives and structure for the rest of the thesis.

1.1.1 Domestic cattle

Cattle (*Bos spp.*) belong to the Bovidae, a large family of wild and domesticated horned, ruminant herbivores in the order Artiodactyla (mammals with an even numbers of toes on each foot; Vaughan et al. 2000). Extant cattle species include: *Bos taurus*, which predominates in Europe, northern Asia, West Africa, and America; *Bos indicus*, which thrive in more arid climes; Bali cattle of South-East Asia (*Bos javanicus*); gayal or mithan (*Bos gaurus*) of north west India; and the domestic yak (*Bos grunniens*; Bradley & Cunningham 1999).

The economical importance of cattle is enormous. In 2009 total beef world exports had a value of US$6, 508,009,000 while the total value of the cattle milk

(whole fresh) exports in the same year accounted for US$3,925,855,00 (FAOSTAT, 2012). There are over 1.42 billion cattle in the world (FAOSTAT, 2012) and the word "cow" is recognized in 539 different languages and dialects (Velten 2007). Livestock production, which uses land both for grazing and for growing animal feed, takes up to 30 % of the ice-free land on the planet (Bonney & Stamp 2008). Livestock now account for about 20 % of the total animal biomass in the world and destruction of natural habitat for cattle farming is a major problem in many developing countries (Bonney & Stamp 2008). Increasing demand for animal food products has encouraged the development of advanced breeding and feeding technology in livestock production with the aim of maximizing productivity and limiting environmental damage. It has been argued this research focus has meant that animal welfare has been largely overlooked (Albright & Arave 1997; Rushen et al. 2007; Bonney & Stamp 2008).

1.1.2 The origins of domestic cattle

Domestic cattle are descended from the wild species, *Bos primigenius*, the extinct wild "ox" or "aurochs" (Bruford et al. 2003; Bradley & Magee 2006; Hall 2008). It is known that *Bos primigenius* ranged throughout much of Eurasia and Northern Africa during the late Pleistocene and early Holocene (Bradley et al. 1996; Edwards et al. 2010). Bulls were markedly larger than modern domestic bulls, with a height of up to 6.5 feet at the shoulder, and longer horns (Zeuner 1963).

Morphological differences in fossilized horn and body shapes have driven some archaeozoologists to classify the aurochs into three major separate subspecies: a Eurasian subspecies (*Bos primigenius primigenius*), a South Asian subspecies (*Bos primigenius namadicus*), and a North African subspecies (*Bos primigenius opisthonomus*) (Clutton-Brock 1989; Bradley & Magee 2006). The first evidence of human contact with aurochs can be seen in European cave paintings during the upper Palaeolithic period (Rifkin 1992). The last aurochs cow died in Poland in 1627 (van Vuure, 2005).

1.1.3 Modern cattle: a domesticated species

Cattle were domesticated between 10,000 and 8000 years ago (Bailey et al. 1996; Bradley & Magee 2006). Until recently, it was thought that *Bos taurus* and *Bos indicus* were different forms of *Bos primigenius primigenius*. However, recent genetic analysis suggests that the domestication process was more complex: there were two domestication events for taurine cattle (from *Bos primigenius primigenius*), once in Eurasia and once in Africa, and a third separate domestication event for zebu cattle (from *Bos primegenius nomadicus;* Bruford et al. 2003).

Domestication implies a significant change in an animal's way of life. Domestic animals are protected from predators, are provided with food, and live in artificial

environments (Stricklin 2001; Zeder 2012). In cattle and many other domesticated species such as goats and sheep, changes in behavioural patterns occur through artificial selection and/or phenotypic plasticity during the domestication process. Nevertheless, Price (1984) argues that although domestication may have altered the threshold and frequency at which some behavioural patterns are expressed, the basic social characteristics of domestic animals remain similar to those of their wild conspecifics or ancestral species.

1.1.4 Feral cattle

Although there are no populations of wild cattle left, observations of feral domesticated cattle can give us insights into the likely behaviour of the ancestral wild species *Bos primigenius*, and help to clarify which traits have remained and which ones have changed/evolved during recent history (Bouissou et al. 2001). The few populations of cattle in the world which are truly feral include those on Amsterdam Island, in the Indian Ocean (Daycard 1990), a population in the south of Spain (Lazo 1994), and a herd in the Orkney islands (Hall and Moore 1986). The two best-studied feral populations of *Bos taurus*, however, are Chillingham cattle, which have inhabited Chillingham Park, Northumberland with minimal interference from man since the 13th century (Hall 1986), and Maremma cattle, which have been protected in the Ponticelli reserve, Italy, without extensive human interactions for more than 1500 years (Lucifero et al. 1977). It seems

reasonable to expect that the behaviour of these breeds more closely resembles that of the original wild cattle than the behaviour of modern breeds in artificial agricultural environments. Comparative studies of modern breeds with these populations as well as with other wild bovids have the potential to shed light on the behavioural patterns which have been altered due to agricultural management.

1.1.5 Social behaviour and grouping

The family Bovidae comprises 14 subfamilies, among which the Bovinae, to which cattle belong. Bovine species, which include cattle, African buffalo (*Syncerus caffer*), bison (*Bison bison*) and yak (*Bos grunniens*), are not territorial (Bouissou et al. 2001). Major features of their social organization include the integration of males and females into mixed herds, precocial young, group defence, social licking and minimal social distance (Estes 1974). It is known that feral bovine populations aggregate in herds of cows and calves that can include mature males.

The social systems of most of the wild African Bovinae are characterized by a dominance hierarchy between adult males (Estes 1974; Vaughan et al. 2000). In feral cattle populations the hierarchical order has been observed both among adult males and also in “bachelor” groups where sub-adult males live together. It has been observed that the dominance relationships between males are less

stable from year to year than those established between females, and that middle-aged males (3-5 years) tend to be the dominant individuals (Bouissou et al. 2001). The social interactions between cows have rarely been described for feral animals. In the Chillingham population, no specific associations between individuals have been found, but strong affinities exist among social "classes" (high-ranking females tend to associate with high-ranking males; Hall 1986). Crèching behaviour, where calves tend to cluster in small groups of similar ages, is a pattern commonly observed in Maremma cattle (Vitale et al. 1986).

1.1.6 Farm cattle

Most of the available information about cattle physiology and behaviour comes from studies of modern domestic breeds kept on farms. Since farm animals are kept in limited enclosures, and under such conditions it is possible to control many variables, they are good models for biological and behavioural studies. Although most studies of domestic cattle are aimed at improving farm productivity (Müller & von Keyserlingk 2006), some fundamental research on farms has helped us to understand various aspects of behaviour, ecology and evolution of domesticated species. In addition, in recent years, applied studies focussing on animal welfare have become increasingly common, and have made significant progress in identifying appropriate measures for balancing the need

for productivity with the need to maintain the welfare of animals living in farms (Boissy & Bouissou 1995; Herskin et al. 2003).

Despite the genetic and physical differences between zebu (*Bos indicus*) and taurine cattle (*Bos taurus)*, the two types can interbreed and produce fertile offspring (Tucker 2009). Zebu cattle are more tolerant of heat than taurine cattle and the two types are intermixed to create a hardy beef animal, common in hot countries like Australia. There are hundreds of breeds of cattle throughout the world, produced through centuries of selective breeding, both within and between the two types of cattle (Buchanan& Dolezal 1999).

In the developing world cattle serve many functions, including food production (both milk and meat), as work animals and to maintain grassland (Tucker 2009). In the industrialized world, specialised breeds dominate milk and meat production. Holstein-Friesian and Jersey cattle are typically used for milk production (Buchanan & Dolezal 1999). There are several common husbandry systems in the dairy industry, but in general dairy cattle are relatively tightly constrained in limited housing systems. On the contrary, beef cattle tend to be kept “free range” in fields (Bazeley & Hayton 2007). Breeds of cattle commonly used for meat production include Angus, Hereford and Charolais. Beef production is often divided into two phases: a) cow-calf operations, where mothers and their

calves are maintained together on a free range pasture for periods of 6 - 8 months after calving, and b) finishing operations, where once the calves are weaned they are concentrated in feedlots, usually on a grain-based diet (Tucker 2009).

1.1.7 Social behaviour

Since cattle are gregarious animals, isolated cattle show clear signs of stress including increased heart rate, vocalizations and defaecation/urination (Rushen et al. 1999). In farms, the group size and composition is determined by the farmers and these groups are typically: a) all adults or all juvenile females (mostly in dairy farms); b) a mix of cows, calves and a few bulls during the breeding season; or c) in feedlots, a mix of both sexes, sometimes castrated or spayed, depending on age and practice within a country (Tucker 2009).

Reproductive activity in a cattle herd usually affects the social grouping. Gestation in cattle lasts for approximately nine months, although breed and sex of calf can affect the exact gestation length (Tucker 2009). Cows do not usually prepare a nest site, but in cattle, most pre-parturient females show a strong tendency to isolate themselves from the rest of the herd (Lidfors et al. 1994; Keyserlingk & Weary 2007). There are examples of other ungulates where there is no such isolation, with mothers giving birth within the herd, most probably

because of the adaptive value of communal defence against predators in wild populations (Estes & Estes 1979). The crèching behaviour which has been observed in feral cattle, where calves tend to cluster in small groups of similar ages, is a pattern commonly observed in free range beef cattle (Bouissou et al. 2001). Sometimes mothers remain in close proximity to these calf groups, potentially acting as a guard (Bouissou et al. 2001). The function of the crèching behaviour is not clear, but it has been hypothesized that it could have an anti-predator function, or it may decrease the negative influence of flies, and/or allow socialization among calves (Bouissou et al. 2001).

Social interactions in cattle can be roughly divided into agonistic, including aggressive acts and responses to aggression (mainly avoidance reactions), and non-agonistic, including in particular allogrooming and sexual behaviour (Bouissou et al. 2001). Aggressive behaviour includes threats such as lowering the head (as though to present horns) and can escalate to physical contact in the form of head butting the head or body of another individual, or head-to-head pushing (Tucker 2009). The most common affiliative behaviour in cattle is allogrooming, or social licking. Social licking between adult cattle is often directed at the neck region of the body, and cattle form grooming partnerships with specific individuals within a group (Tucker 2009). Licking of calves by their mothers is a very important behaviour immediately after birth. A cow typically spends 30 % - 50 % of the first hour after birth licking her calf (Edwards & Broom 1982). In precocial species, it has been shown that licking plays an important role

in the establishment of the mother-offspring bond (Edwards & Broom 1982). Additionally, several other physiological functions of licking have been suggested, such as cleaning and drying the calf's coat, and stimulating breathing, circulation, urination and defecation (Metz & Metz 1986; Keyserlingk & Weary 2007).

Positive and aggressive social interactions among cattle are known to lead to the establishment of dominant-subordinate relationships within the herd (Tucker 2009). These relationships can affect access to resources such as food, lying space, shelter and oestrous females. Aggressive interactions are common when unfamiliar individuals are mixed together, but generally decline over time as animals establish a dominance hierarchy (Bouissou et al. 2001). Individual characteristics, such as the presence of horns and body size, can influence social success. It has been shown that among similar size cattle, cows with horns were dominant over cows without horns 85 % of the time (Bouissou 1972).

1.2 Communication in cattle

1.2.1 Visual communication

Visual signals are one of the most important means of communication in cattle. Grazing mammals have wide-set eyes and panoramic vision, an adaptation for

survival in the face of the risk of predation (Bouissou et al. 2001). Their angle of vision is approximately 320^{o}. Colour vision has been demonstrated by operant conditioning experiments (Riol et al. 1989) and has been subsequently corroborated using elecroretinogram flicker photometry (Jacobs et al. 1998). Interestingly, some studies carried out in adult cattle have shown that they are able to identify conspecifics and even different breeds efficiently only by visual discrimination. Using an experimental design with 2D images from cow breeds with different coat patterns, it has been shown that cattle use visual discrimination in coat patterns, and familiarity improves their performance in recognition (Coulon et al. 2007, 2009). The ability to communicate through facial expressions is limited in cattle, especially compared with horses (Bouissou et al. 2001). In contrast, the mobility of the head allows displays in which its position with respect to the body plays an important role, for example in aggressive or submissive displays (Scholoeth 1958). The position of the tail is also known to indicate a cow's mood and activity (Albright and Arave 1997).

1.2.2 Olfactory communication

A large number of odoriferous glands (interdigital, infraorbital, inguinal, sebaceous glands, etc.) are present in cattle, which suggests the importance of olfaction in their social life (Bouissou et al. 2001). Indeed, there is evidence that olfactory cues are important in social, sexual and maternal behaviour in cattle

(Bouissou et al. 2001). Olfaction is of importance in social relationships as it contributes to individual recognition, albeit only over short distances. It has been shown that cattle can be trained to distinguish between conspecific individuals through olfactory cues alone (Baldwin 1977). As in other animals, cattle also appear to communicate their psychological state, especially when frightened or stressed, by means of pheromones. Interestingly, experiments using stressed individuals and urine from stressed conspecifics have shown that cows are slower to learn tasks in presence of stressed conspecifics and slower to approach the food in presence of urine from stressed conspecifics (Boissy et al. 1998).

1.2.3 Vocal communication

The sense of hearing is much more sensitive in cattle than in humans (Heffner 1998). Cattle are able to perceive a more extensive range of frequencies (from 23 to 37,000 Hz) and their sensitivity to high and low frequencies is much better than in humans, with a maximal sensitivity at 8000 Hz (Heffner 1998). The detection of this range of frequencies allows cattle to recognise threats from predators at great distances, and to some extent to locate the source of noises, although cattle and other domestic species like goats are relatively inaccurate localizers of sounds (Heffner & Heffner 1992). Cattle hearing also enables them to individually identify their own offspring calls (Barfield et al. 1994; Marchant-Forde

et al. 2002), but the ability of offspring to identify their own mothers' calls has not previously been tested.

Cattle, like many other gregarious mammals, use vocalizations to communicate. Very little research on vocal communication has been done in cattle. There have been some attempts to describe cattle vocalizations, but most of the research done in this field has been limited to descriptive accounts of the different vocal signals produced by individuals. For example, Schleoth (1961) reported that there were eleven different vocalizations in Camargue cattle. Unfortunately there was no acoustical analysis done. Kiley (1972) created a complex phonetic classification using sonograms and described six different types of calls in domestic cattle. However, the Kiley (1972) classification is largely descriptive and her call types are difficult to interpret in the field. Nevertheless, it is probable that cattle do produce distinctly different vocalizations in different contexts or internal/emotional states, that these differences are meaningful to other individuals who hear them, and that calls have characteristics which differ between individuals.

1.3 Individual consistency of behavioural patterns in cattle

The reasons why individuals (animals or humans) differ in the way they react to potential risks, handle novelty, or interact with conspecifics, have been intensively researched over the past decade. The scientific community has

become particularly interested in individual differences in behaviour that are consistent across time and/or across contexts, as evidenced by the rapidly growing literature on animal personality, temperament, coping styles, and behavioural syndromes (Reale et al. 2007; Sih & Bell 2008; Stamps & Groothuis 2010). Behavioural differences among individuals, which are often highly structured, stable over time and correlated across different situations and contexts (Sih et al. 2004; Bell et al. 2009), are a common feature of animal populations (wild or domestic) in a diverse range of species across the animal kingdom (Gosling 2001; Reale et al. 2007).

Variation among individuals in observed temperament is considered to reflect differences in fear, social motivation, exploratory motivation or a combination of all of these factors (Mackay and Wood-Gush 1980; Boissy and Bouissou 1995). Animal personality or temperament has been shown to influence the productivity of cattle (Müller & von Keyserlingk 2006). Consequently, a number of studies have attempted to develop tests to evaluate cattle temperament, with the applied aim of understanding variation among individuals in how easy they are to handle/manage in a farm environment, and in productivity. Examples of these tests include the social separation test (de Passille´ et al. 1995; Müller & Schrader 2005), the flight speed test, the results of which correlate with weight gain (e.g. Burrow et al. 1988; Burrow and Dillon 1997; Petherick et al. 2002; Müller & von Keyserlingk 2006), and the fearfulness test (Breuer et al. 2000; Hemsworth et al.

2000). While these tests have been designed with the explicit objective of improving animal productivity and/or welfare of cattle, they indicate that consistent individual-specific differences in responses to stimuli are important in the behavioural ecology of this species. Nevertheless, the behaviours with direct relevance to agricultural practice (e.g. handling, productivity), which have been the focus of research into cattle temperament, do not map onto, or reflect the entire scope of, the dimensions of personality which have been studied more widely in fundamental behavioural research and which are known to play an important role in the interplay between social interactions, life history and individual fitness (Reale et al. 2007; Dingemanse et al. 2009). Additionally, most existing studies of cattle behaviour have been done on animals in very constrained conditions (e.g. confined in cattle sheds) which probably do not reflect very closely the conditions in which cattle behaviour evolved.

1.4 Overall objectives and thesis structure

The general objective of my thesis was to investigate cattle behaviour, with a focus on vocal communication (Chapters 2 and 3) and behavioural individuality (Chapters 2 and 4). More specifically, in Chapter 2 my aim was to describe vocalisations in free range cattle formally and quantitatively, and to establish the extent to which they are individually acoustically distinctive. A comprehensive study of the attributes of cattle vocalisations is required if the potential for the study of vocal communication to improve our understanding of animal welfare and agricultural production is to be realised. In Chapter 3, I investigate mother-offspring individual recognition of vocalisations using playback experiments. A major aim of this chapter was to establish whether mother-offspring recognition in cattle is unidirectional or bidirectional, and hence whether cattle behave in a way which is consistent with the long-standing hypothesis that in ungulates the proximity of offspring to their mothers while they are foraging determines the directionality of vocal communication in the months after calves are born (Torriani el al. 2006; Sebe et al. 2007; Briefer & McElligott 2011). In Chapter 4, I investigate the individual consistency in behavioural responses towards different visual and auditory stimuli. I present the results of a series of experiments testing individual animals' responses to the presentation of three different novel objects, and to playbacks of familiar and unfamiliar hetrospecific animals. These experiments allowed me to test for individual consistency, and to score behavioural responses as relatively bold or shy in two widely different contexts.

Finally, in Chapter 5, I integrate the findings from the preceding chapters, aiming to find correlations among the acoustic characteristics of individuals' contact calls, their ability to identify own mother or offspring, and their behavioural responses when visual and auditory stimuli were presented. I discuss the relevance of the results obtained for the fields of vocal communication and individuality in animal behaviour ("personality" and correlations across different contexts), considering additionally the relevance of my research to farm animal welfare.

1.5 References

Albright, J. L., & Arave, C. W. 1997. The behaviour of cattle. CAB international, Wallingfrod, UK, 306 pp.

Bailey, J. F., M. B. Richards, V. A. Macaulay, I. B. Colson, I. T. James, D. G. Bradley, R. E. Hedges, & Sykes, B. C. 1996. Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species. Proceedings of the Royal Society of London B Biological Sciences 263: 1467–1473.

Baldwin, B. A. 1977. Ability of goats and calves to distinguish between conspecifics urine samples using olfaction. Applied Animal Ethology 3, 145-150.

Barfield, C. H., Tang-Martinez, Z., Trainer, J.M. 1994. Domestic calves (*Bos taurus*) recognize their own mothers by auditory cues. Ethology 97, 257-264.

Bazeley, K., & Hayton, A. 2007. Practical cattle farming. The Crowood press, Wiltshire, UK. 224 pp.

Bell, A.M. Hankison, S. J., & Laskowski, K. L. 2009. The repeatability of behaviour: a meta analysis. Animal Behaviour 77, 771–783

Boissy, A., Terlow, C., & Le Neindre, P. 1998. Presence of cues from stressed conspecifics increases reactivity to aversive events in cattle: evidence for

the existence of alarm substances in urine. Physiology and Behaviour 63, 489-495.

Boissy, A., Bouissou, M. F., 1995. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Applied Animal Behaviour Science 46, 17–31.

Bonney, R., & Stamp D. M. 2008. The future of animal farming. In: Stamp D. M. & Bonney, R. (Eds.) The future of animal farming. Renewing the ancient contract. Blackwell publishing. Oxford, UK. pp. 1-4.

Bouissou, M. F. 1972. Influence of body weight and presence of horns on social rank in domestic cattle. Animal Behaviour 20, 474-477.

Bouissou, M. F., Boissy, A., Le Neindre, P., & Veissier, I. 2001. The social behaviour in cattle. In: Keeling, L. J., & Gonyou, H. W. Social behaviour in farm animals. CABI Publishing. Oxon, UK. pp. 113-145.

Bradley, D. G., MacHugh, D. E., Cunningham, P., & Loftus, R. T. 1996. Mitochondrial diversity and the origins of African and European cattle. Proceedings of the National Academy of Sciences 93, 5131–5135.

Bradley, D. G., & Cunningham, P. 1999. Genetic aspects of domestication. In: Fries, R., & Ruvinsky, A. The genetics of cattle. Cabi Publishing, UK. pp. 15-31.

Bradley, D.G., & Magee, D.A. 2006. Genetics and the origins of domestic cattle. In: Zeder, M.A., Bradley, D.G., Emshwiller, E., & Smith, B.D. (Eds.) Documenting domestication: new genetic and archaeological paradigms. London: University of California Press. pp 317–328.

Breuer, K., Hemsworth, P.H., Barnett, J.L., Matthew, L.R., & Coleman, G.J. 2000. Behavioural responses to humans and the productivity of commercial dairy cows. Applied Animal Behavioural Science 66, 273-288.

Briefer, E., McElligott, A. G. 2011. Mutual mother-offspring vocal recognition in an ungulate hider species (*Capra hircus*). Animal Cognition 14, 585-598.

Bruford, M.W., Bradley, D. G., & Luikart, G. 2003. DNA markers reveal the complexity of livestock domestication. Nature Reviews Genetics 4, 474-477.

Buchanan, D. S., & Dolezal, S. L. 1999. Breeds of cattle. In: Fries, R., & Ruvinsky, A. The genetics of cattle. Cabi Publishing, UK. pp. 697-695.

Burrows, H.M., & Dillon, R.D. 1997. Relationship between temperament and growth in a feedlot and commercial carcass traits in *Bos indicus* crossbreds. Australian Journal of Experimental Agriculture 37:407-411.

Coulon, M., Deputte B.L., Heyman, Y., Richard, C., & Delatouch, L. 2007. Visual discrimination by heifers (*Bos taurus*) of their own species. Journal of Comparative psychology 121, 198-204.

Coulon, M., Deputte B.L., Heyman, Y., & Baudoin C. 2009. Individual recognition in domestic cattle (*Bos taurus*): Evidence from 2D- Images of heads from different breeds. PLoS One 4, 1-8.

Clutton-Brock, J. 1989. Cattle in ancient North Africa. In: Clutton-Brock, J. (Ed.) The walking larder: Patterns of domestication, pastoralism and predation. Unwin Hyman London, UK. pp. 201–206.

Daycard, L. 1990. Structure sociale de la population de bovins sauvages de l'ile d'Amsterdam, sud de l'Océan Indien. Revue d'Ecologie (La Terre et la Vie) 45, 35-53.

de Passille´, A.M., Rushen, J., Martin, F., 1995. Interpreting the behaviour of calves in an open-field test: a factor analysis. Applied Animal Behavioural Science 45, 201–213.

Dingemanse, N.J., Kazem, A.J.N., Reale, D., & Wright, J. 2009. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology and Evolution 25, 81-89

Edwards, S. A., Broom, D. M. 1982. Behavioural interactions of dairy cows with their newborn calves and the effects of parity. Animal Behaviour 30, 525-535.

Edwards, C. J., Magee, D. A., Park, S. D. E., McGettigan, P. A., Lohan, A. J., Murphy, A., Finlay, E. K., Shapiro, B., Chamberlain, A. T., Richards, M. B.,

Bradley, D. G., Loftus, B. J., & MacHugh, D. E. 2010. A Complete Mitochondrial Genome Sequence from a Mesolithic Wild Aurochs (*Bos primigenius*). A Peer Reviewed, open access journal ONE 5, 1-13.

Estes, R. D. 1974. Social organization of the African Bovidae. In: Geist, V. & Walther, E. (Eds.) The behaviour of ungulates and its relation to management IUNC Publication new series 24, Morges, Switzerland. pp. 166-205.

Estes, R. D., & Estes, R. K. 1979. The birth and survival of wildebeest calves. Zeitschrift für Tierpsychologie 50, 45-95.

Food and Agriculture Organization of the United Nations statistics (FAOSTAT). http://faostat.fao.org/site/573/DesktopDefault.aspx (World trade export value for cattle milk and meat (last update 2009); and world cattle stocks (last update 2010)) (accessed, 24 August 2012).

Gosling, S.D. 2001. From mice to men: what can we learn about personality from animal research? Psychology Bulletin 127, 45–86.

Hall, S. J. G. 1986. Chillingham cattle: dominance and affinities and access to supplementary food. Ethology 71, 201-215.

Hall, S. J. G. 1986. Feral cattle of Swona, Orkney Islands. Mammal Review 16, 89-96.

Hall, S. J. G. 2008. A comparative analysis of the habitat of the extinct aurochs and other prehistoric mammals in Britain. Ecography 31, 187-190.

Heffner, H. E. 1998. Auditory awareness. Applied Animal Behaviour Science 57, 259–268.

Heffner, R. S., & Heffner, H. E. 1992. Hearing in large mammals: Sound-Localization Acuity in Cattle *(Bos taurus)* and Goats *(Capra hircus)*. Journal of Comparative Psychology 106, 107-113.

Hemsworth, P.H., Coleman, G.J., Barnett, J.L., & Borg, S. 2000. Relationships between human-animal interactions and productivity of commercial dairy cows. Journal of Animal Science 78, 2821-2831.

Herskin, M. S., Munksgaard, L., & Kristensen, A.M. 2003. Testing responses to novelty in cattle: behavioural and physiological responses to novel food. Animal Science 76, 327-340.

Jacobs, G. H., Deegan, J. F., & Neitz, J. 1998. Photopigment basis for dichromatic color vision in cows, goats, and sheep. Visual Neuroscience 15, 581-584.

Keyserlingk, M.A.G., & Weary, D.M. 2007. Maternal behaviour in cattle. Hormones and Behaviour 52, 106-113.

Kiley, M., 1972. The vocalisations of ungulates, their causation and function. *Z*eitschsrift für Tierpsychology 31, 171-222.

Lazo, A. 1994. Social aggregation and the maintenance of social stability in a feral cattle population. Animal Behaviour 48, 1133-1141.

Lidfors, L. M., Jensen, P., & Algers, B. 1994. Suckling in free-ranging beef cattle-Temporal patterning of suckling bouts and effects of age and sex. Ethology 98, 321-332.

Lucifero, M., Janella, G. C., & Secchiari, P. 1977. Origini, evoluzione, miglioramento e prospettive delle razza bovina Maremmana. Edagricle, Bologna.

Mackay, P.C., & Wood-Gush, D.G. 1980. The responsiveness of beef calves to novel stimulation: an interaction between exploration and fear. Applied Animal Ethology 6, 383–384.

Marchant-Forde, J. N., Marchant-Forde, R. M. & Weary, D.M. 2002. Responses of dairy cows and calves to each other´s vocalisations after early separation. Applied Animal Behaviour Science 78, 19-28.

Metz, J., & Metz, J.H.M. 1986. Maternal influence on defecation and urination in the newborn calf. Applied Animal Behavioural Science 16, 325–333.

Müller, R., Schrader, L., 2005. Behavioural consistency during social separation and personality in dairy cows. Behaviour 142, 1295–1312.

Müller, R., von Keyserlingk, M. A. G. 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle.

Petherick, J.C., R.G. Holroyd, V.J., Doogan, & Venus, B. K. 2002. Productivity, carcass and eat quality of lot fed *Bos indicus* cross steers grouped according to temperament. Australian Journal of Experimental Agriculture 42, 389-398.

Price, E. O. 1984. Behavioural aspects of animal domestication. Quarterly Review of Biology 59, 419-422.

Réale, D., Reader, S. M., Sol, D., McDougall, P.T., & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. Biology Reviews 82, 291-318.

Rifkin, J. 1992. Beyond beef: The rice and fall of the cattle culture. London, pp. 185-230.

Riol, J. A., Sanchez, J. M., Eguren, V. G., & Gaudioso, V. R. 1989. Colour perception in fighting cattle. Applied Animal Behaviour Ethology 4, 43-54.

Rushen, J., Boissy, A., Terlouw, E. M. C., & Passill[e, A. M. B. 1999. Opioid peptides and behavioural and physiological responses of dairy cows to social isolation in unfamiliar surroundings. Journal of Animal Science 77, 2918-2924.

Rushen, J., Passillé A.M., von Keyserlingk, M.A.G., & Weary, D.M. 2007. The Welfare of Cattle. Springer, USA, 310 pp.

Schloeth, R. 1958. Le cycle annuel et le comportement social du taureau de Camargue. Mammalia 22, 121-139.

Schloeth, R. 1961. Das sozialleben des Camargue Rindes. Zeitschrift für Tierpsychologie 18, 574-627.

Sebe, F., Nowak, R., Poindron, P., & Aubin T. 2007. Establishment of vocal communication and discrimination between ewes and their lamb in the first two days after parturition. Developmental Psychobiology 49, 375-386.

Sih, A., & Bell, A.M. 2008. Insights for behavioural ecology from behavioural syndromes. Advances in the study of behaviour **38,** 227-281.

Sih, A., & Bell, A.M., & Johnson C. J. 2004. Behavioural syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution 19, 372-378.

Stricklin, W. R. 2001. The evolution and domestication of social behaviour. In: Keeling , L.J. & Gonyou, H. W. (Eds) Social behaviour in farm animals. CABI Publishing, Oxon, UK. pp. 83-110.

Stamps, J., & Groothuis, T.G. 2010. The development of animal personality: relevance, concepts and perspectives. Biological Reviews 85, 301-325.

Torriani, M. V. G., Vannoni, E. & McElligott, A. G. 2006. Mother-young recognition in an ungulate hider species: a unidirectional process. American Naturalist 168, 412-420.

Tucker, C.B. 2009. Behaviour of cattle. In: Jensen, P. (Ed.) The Ethology of domestic animals. 2nd edition. CABI, UK. pp. 151-160.

Van Vuure C. 2005. Retracing the Aurochs: History, Morphology and Ecology of an extinct Wild Ox. Pensoft, Sofia, Bulgaria, 432 pp.

Vaughan, T. A., Ryan, J. M., & Czaplewski N.J. 2000. Behaviour. Mammalogy. 4th ed. Thompson learning, U.S.A.

Velten, H. 2007. Cow. Reaktion boks, London UK.

Vitale, A. F., Tenucci, M., Papini, M., & Lovari, S. 1986. Social behaviour of the calves of semi-wild Maremma cattle, *Bos primigenius taurus*. Applied Animal Behaviour Science 16, 217-231.

Zeder, M. A. 2012. Pathways to Animal Domestication. In: Biodiverity in Agriculture: Domestication, Evolution, and Sustainability. Gepts, P., Famula, T. R., Bettinger, R. L., Brush, S. B., Damania, A. B., McGuire, P. E., & Qualset, C. O. (Eds.) Cambridge University Press, UK. pp. 227-259.

Zeuner, F. E. 1963. A history of domesticated animals. Hutchison, London, UK.

CHAPTER TWO

Acoustic characterization of cattle vocalisations

2. Acoustic characterization of cattle vocalisations

2.1 Introduction

Vocalisations play a key role in a wide range of contexts in communication among vertebrates, both within and among species. It is widely known that vocalisations are used in sexual contexts (McComb 1991), to advertise the ownership of a territory (McComb et al. 1994), cooperation and for individual recognition in gregarious species (Charrier et al. 2010). Although cattle are domesticated species, and despite the fact that cattle behaviour and welfare are fairly well researched, very few studies have considered their vocal behaviour. What we do know about cattle vocalisations mostly comes from a study by Kiley (1972), which provides a useful attempt to classify and characterise types of cattle vocalisation. The value of this study, however, is rather limited because it was conducted before modern techniques for sound recording and analysis were widely available, and before our understanding of the mechanistic basis of variation in sound production in vertebrates was transformed by the introduction of the source-filter framework (Fant 1960; Titze 1994; Taylor and Reby 2010). To date, the source-filter framework has never been applied in the study of cattle vocalization. In this chapter, in order to identify in detail the acoustic features of cattle vocalisations, and to determine whether they are individually distinctive, I use modern techniques to systematically and quantitatively describe and

compare cow and calf contact calls made under semi-natural conditions in a free-ranging herd. I analyse and discuss the data in the context of source-filter theory of sound production.

2.1.1 Animal communication

The study of animal communication is fundamental in order to understand social behaviour. Nevertheless, to understand how communication systems in the animal kingdom work is not easy and it has even been difficult to agree upon a working definition of "communication". However, there is a general consensus among researchers that communication involves the provision of information by a sender to a receiver (Bradbury and Vehrencamp 1998). The acquisition and the use of information helps receivers to anticipate and respond appropriately to events, and hence to maximise their fitness (Owings et al. 1997). Similarly, the provision of information helps senders to influence the behaviour of other individuals in ways which reduce the fitness cost or increase the benefit of those behaviours to the sender. The vehicle that provides the information is defined as a signal, which is typically (but not exclusively) visual, olfactory or vocal (Bradbury and Vehrencamp 1998).

There are several ways in which a signal can be emitted by a sender and they can be classified by the contexts in which they occur. Thus, there are conflict

resolutions signals (Robertson 1986), territorial signals (Harrington & Mech 1979), sexual signals (mate attraction and courtship; McComb 1991), parent-offspring signals (Sebe et al. 2007), social integration signals (McComb et al. 2003), environmental signals (Linge et al. 2007) and autocommunication signals (Kalko 1995). Signalling by any modality (e.g. sound, sight, touch, electrical or chemical) has an energetic cost. It has been hypothesised that signalling has often been favoured by natural selection because it substitutes for behaviour that is even more energetically costly. For example, fighting could be more likely than communicating to result in injury or attract predators (Owings & Morton 1998). For a sender, the function of sending a signal is to increase the chances that the receiver will select that action most beneficial for the sender; for a receiver, the function of responding to a signal is to increase its own chances that it chooses the action best for it (Bradbury and Vehrencamp 1998).

Signals can evolve to be reliable or honest if there are cost and constraints to the sender that make conveying truthful information a more optimal strategy than lying (Zahavi & Zahavi 1997; Reby & McComb 2003). Vehrencamp (2000) defined signal types based on these costs and constraints where "index" signals cannot be faked because of physical or physiological constraints, "handicap" signals are more reliable because of production costs or increased vulnerability to attack by inter- or intraspecific receivers, and "conventional" signals, while not risky or costly to produce, are kept honest by the threat of receiver retaliation (Wyman et

al. 2008). When acoustical individual recognition benefits both senders and receivers, senders should develop distinctive signals of identity and receivers should develop accurate mechanisms for recognition of the differences in these signals among individuals (Tibbets and Dale 2008).

2.1.2 Vocal communication in mammals

Vocal communication using simple or complex sounds is common in vertebrates (Simmons 2003). Particularly in mammals, acoustic signals can encode different types of information, and thus are used in many forms of social interactions (Fischer et al. 2002; Theis et al. 2007; Vannoni 2007; Taylor et al. 2009). The frequencies of mammal vocalizations from different species range over nearly five orders of magnitude, from 9 Hz in some whales (Mellinger & Clark, 2003) to above 110,000 Hz in some bats (Jones 1999).

There are many different contexts in which terrestrial mammals vocalize. Vocal communication facilitates several types of essential interaction among individuals of the same species (Owings & Morton 1998). First, vocalisations are used as indicators of mate quality, dominance and readiness to mate in sexual interactions, both among males (e.g. red deer, *Cervus elaphus*, Reby et al. 2005; in fallow deer, *Dama dama*, McElligott et al. 2006; Vannoni & McElligott 2008) and between males and females (e.g. in macaques, *Macaca sylvanus*, Semple &

McComb 2000; in red deer, Charlton et al. 2007). They can also be critical in partner and mother-offspring recognition (e.g. contact calls in fallow deer, Torriani et al. 2006, in sheep, *Ovis aries,* Sebe et al. 2007, in goats, *Capra hircus,* Briefer & McElligott 2011a). In social groups, vocal communication allows individuals to keep in contact with others in their own group even if when they are widely separated (e.g. in wolves, *Canis lupus;* Tooze et al. 1990; in lions, *Panthera leo*, McComb et al. 1994; in elephants, *Loxodonta africana*, McComb et al. 2003), and facilitates the dissemination of information about shared risks (e.g. alarm calls in the vervet monkey, *Cercopithecus aethiops*; Seyfarth et al. 1980), and coordination of defence against predators (e.g. distress calls in mule deer fawns, *Odocoileus hemiounus* & *O. virginianus*; Linge et al. 2007).

Vocalizations can carry important information about the sender to the receiver. They can encode individual identity, which is likely to be particularly important in individual recognition (e.g. between mothers and offspring) when individuals range widely, and hence visual or olfactory signals are not available (Searby & Jouventin 2003). There is also good evidence that vocal cues can inform receivers about the location of the caller, and its physical attributes (McComb & Reby, 2005). For example red deer (*Cervus elaphus*) roars are used to infer body mass, age and fitness (Reby & McComb 2003), and goat (*Capra hircus*) kid calls reveal information about their sex, age, and body weight (Briefer & McElligott 2011a).

Source-filter theory of vocal production

Early research on mammal vocal communication, and applied studies of vocalisation in the context of animal welfare and productivity, have generally focused on the most obvious (to the human ear) and easily measured parameters of vocalizations such as calling rate loudness, and aspects of behavioural interactions between signaller and receiver. These early studies often relied on the descriptive and/or non-quantitative classification of calls into types, according to different contexts (McComb 1991; Owings & Morton 1998; Weary & Fraser 1995; Byrne & Suomi 1999; McElligott & Hayden 1999; Marchant et al. 2001). The application of the source-filter theory (Fant 1960; Titze 1994) and the development of new signal analysis techniques have led to significant advances in our understanding of this subject. The modern approach allows researchers to describe in detail the structure and variation of the acoustic parameters present in animal vocalizations, to link vocal production with the acoustic structure, and finally, to understand to what extent calls vary between individuals and between contexts (Taylor & Reby 2010).

Originally, the source-filter theory was created in order to analyse human speech (Fant 1960; Titze 1994). Speech researchers determined that the production of the voiced signals that form human speech is a two-stage process, where the vocalizations are generated by vibrations of the vocal folds (the “source”,

determining the fundamental frequency, 'F0') and subsequently filtered by the supralaryngeal vocal tract (the “filter”, producing amplified frequencies called 'formants'; Fant 1960; Titze 1994). Although vocal communication in other mammal species is qualitatively different from human speech, our mechanisms of vocal production are largely shared (Titze 1994; McComb & Reby 2009). Researchers realised that it is possible, therefore, to generalise the source-filter theory to other vertebrates, and this stimulated bioacoustics research on a wide array of species (Newton-Fischer et al. 1993; Fitch 1997; Owren et al. 1997; Reby et al. 1998; Riede & Fitch 1999; McComb et al. 2003; Torriani et al. 2006; Briefer & McElligott 2011a).

According to the source-filter theory of voice production (Fant 1960; Titze 1994), the source is located specifically in the larynx and all sub-laryngeal and laryngeal structures, and includes the production of the signal that is generated by the vibrations of the vocal folds. The vocal folds consist of three layers: muscle, vocal ligament and epithelium. The vocal folds and the space between them form the glottis (Taylor & Reby 2010). The source shapes certain characteristics of the vocalization such as the fundamental frequency (determined specifically by the rate of opening and closing of the glottis), the duration of the call, the periodicity of the signal, and its spectral slope. It has been shown that the source determines the presence of phenomena associated with non-linear dynamics such as subharmonics (additional harmonics visible in the spectrum beneath the

fundamental frequency; for example, in chimpanzees (*Pan troglodytes*): Riede, et al. 2004), and biphonation (two independent fundamental frequencies, as in African wild dogs (*Lycaon pictus*); Wilden et al. 1998). These parameters can vary between individuals as a result of differences in the way that larynx is operated, or because of variation in the morphology of callers (Reby & McComb 2003; McComb & Reby 2005).

In the supra-laryngeal vocal tract, which is defined as the tube that links the larynx to the openings (mouth and nose), and from which the sound radiates to the environment (Titze 1994), certain frequencies in the source spectrum are selectively amplified or "filtered". The acoustic characteristics of the filter determine the frequencies and bandwidths of the formants, which in turn describe the shape of the spectral envelope. Formant frequencies are determined by the length and shape of the cavities of the vocal tract, pharynx, mouth and nasal cavities (McComb & Reby 2005). It has been shown that variation in the source-filter parameters encodes individual identity in many species of large mammals. For example, differences in fundamental frequency appear to encode individual identity in wolves (*Canis lupus*; Tooze et al. 1990), and differences in formants seem to be important in individuality coding for fallow deer (*Dama dama*; Reby et al. 1998; Vannoni & McElligott 2008), African elephants (*Loxodonta africana*; McComb et al. 2003) and rhesus macaques (*Macaca mulatta*; Rendall et al. 1998).

2.1.3 Vocal communication in cattle

Vocal communication in cattle has not been examined in detail in the context of the source-filter theory. Given what we know about other mammals and ungulates (Fitch 1997; Reby & McComb 2003; Briefer & McElligott 2011a,b), it is highly likely that characteristics of cattle vocalizations provide information about the caller, such as age, sex and individuality, to conspecifics. It has also been proposed that vocalizations in cattle may signal the physiological and emotional state of the calling animal (Watts & Stookey, 2000; Marchant-Forde et al. 2002).

Some researchers have already suggested that individual cattle and different cattle populations have distinctive calls (Kiley 1972; Hall et al. 1988). For example, in a study of Chillingham cattle, Hall et al. (1988) showed that bulls have complex and loud vocalizations in comparison with other breeds of *Bos taurus*. It has been hypothesised that such vocal complexity might be a result of young bulls being able to practise, without attracting the attention of predators. Because breeding occurs year-round in Chillingham cattle, such calls are unlikely to evoke as much aggression from mature bulls as they would if there was a rut (Hall et al. 1988). However, this and other evidence about individuality in cattle vocalisations is largely anecdotal, and comes from studies that did not use the source-filter framework, and which were not comprehensive in their consideration of the acoustic characteristics of cattle vocalisations.

The first acoustic study on cattle was Kiley's (1972) work, which attempted to classify cattle, pig (*Sus scrofa*) and horse (*Equus caballus*) calls. She mentioned the existence of different types of calls in different behavioural contexts. She identified the five most distinctive “syllables” present in domestic cattle vocalizations (from recordings in cows, bulls and some calves on dairy and beef farms), and she created a classification of the main types of calls she observed. Kiley (1972) for the first time measured some acoustic parameters that she analysed using spectrograms from domestic cattle. Thus, the difference among syllables was based on differences in frequencies, amplitude and tonality. The differences among the types of calls were mainly explained in terms of the way that the animals produced the call (e.g. with full open mouth, closed mouth, etc.). For example, she described the "mm" call, as being produced with the mouth closed, with a low fundamental frequency of 50 - 125 Hz. And she also identified this type of call as a contact call from the mother to her calf. Although this was a pioneering acoustic study of cattle vocalizations, Kiley’s (1972) classification was limited by the technologies for sound capture and processing that were available at the time. She relied on some measurements that she called "subjective", such as the level of excitement of the animal, which she described as being related to the fundamental frequency of the call.

Apart from Kiley's (1972) paper, there is no other published fundamental research into the acoustic characteristics of cattle vocalizations. However, there

are some studies that have measured acoustic features of cattle vocalizations in the context of animal welfare. It has been shown that calves fed according to conventional management (i.e. twice daily for a total of 5 l during 24 hrs) with milk produced a higher call rate with and higher fundamental frequency than calves fed with more than 5 l of milk in 24 hrs (Thomas et al. 2001). Similarly, it has been demonstrated that the vocalizations of a single cow under two different psychological stress conditions, such as being hungry and separated from her calf, are acoustically different (Ikeda & Ishii, 2008). Calls produced by a cow after separation from her calf had lower formant frequencies than those produced under the hunger condition (Ikeda & Ishii, 2008). It has also been widely reported that cows and calves perform more vocalizations after being separated from each other (Weary & Chua 2000). Vocalizations after separation are usually associated with an increase in locomotion and heart rate, which presumably is due to stress (Stehulová et al. 2008). Other studies have evaluated the vocal responses of animals in regard to different farming procedures. For example, it has been observed that there is an increase in the call rate of calves after iron-branding, and it was also shown that branded calves have a higher fundamental frequency, a higher maximum frequency and a higher peak sound level than non-branded calves (Watts & Stookey 1999). Similarly, an increment in the vocalization rate has been reported in cows during handling in the forcing pen, stunning box and single file race of commercial plants when an electrical prod was used excessively (Grandin 1998). These applied studies have demonstrated the potential for the

analysis of cattle vocalisations to provide insights into animal welfare (Watts & Stookey 2000; Manteuffel et al. 2004). In order to develop robust acoustic indicators of animal welfare in cattle, however, it is crucial to have a thorough basic characterization of the acoustical features of vocalizations.

2.2 Objectives of this chapter

The aim in this chapter was to determine the specific acoustic characteristics of cattle vocalizations using modern approaches of analysis, in the context of source-filter theory. To do this, I recorded natural occurring calls from cows and calves in a commercial herd maintained in semi-natural conditions with minimal interference from the owner. I performed an extensive acoustic analysis to indentify the different types of calls between mother and offspring, the differences between calls made by cows and calves, and the extent to which the calls were individually identifiable.

Recognition is required in almost all social behaviours. Individual recognition includes a wide range: including self, kin, mate, gender, neighbour, rival, friend, species, predator, and prey (Tibbetts & Dale 2007). Due to the likely importance of individual recognition and vocalisations in mother-offspring communication in cattle, and because the majority of calls made in free-range herds in farm environments are between cows and calves, I focus here and in the next chapter on the characteristics and function of mother-offspring contact calls.

2.3 Methods

2.3.1 Subjects and study site

The study was carried out with two cattle herds (Herd 1: n = 21 adult females; Herd 2: n = 23 adult females) situated in two separated groups of fields on a farm in Radcliffe on Trent (52° 56′ 44′′N, 1° 02′ 62′′W), Nottinghamshire, UK (Fig. 2.1), from February of 2010 to December 2010.

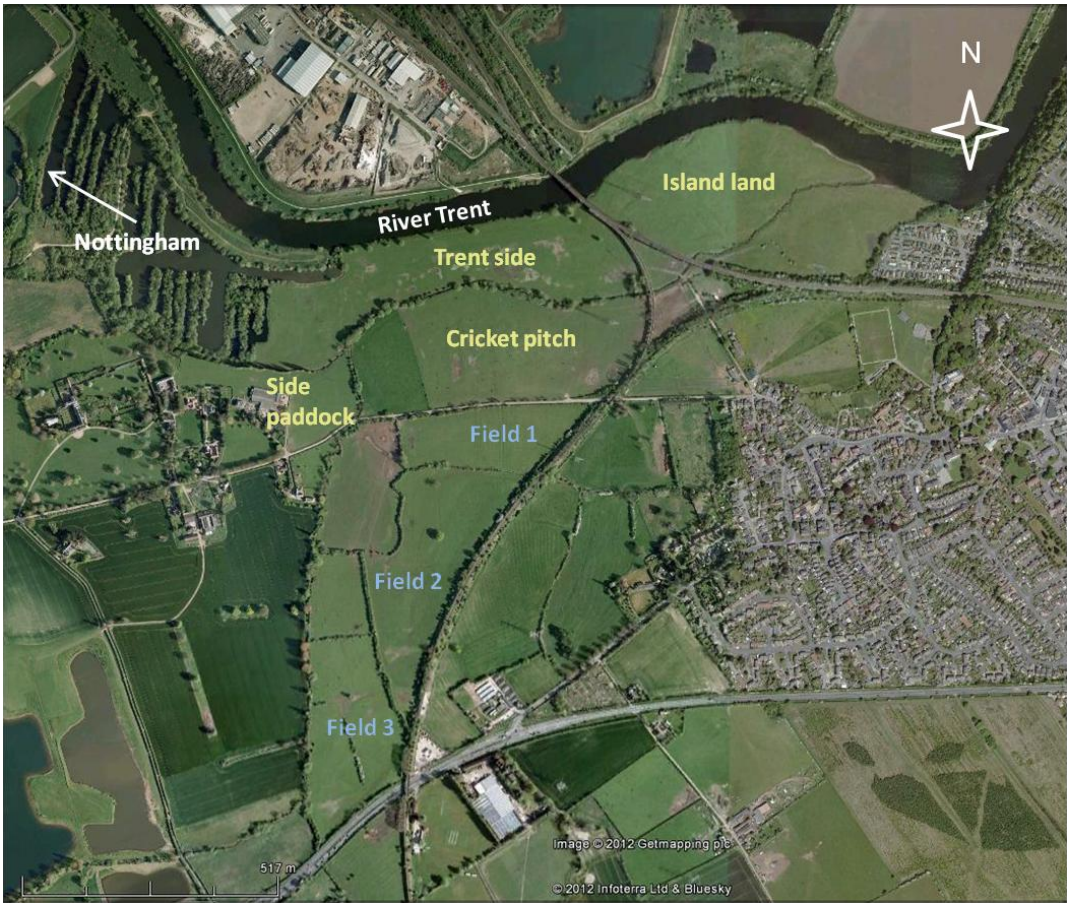

Figure 2.1 The location of the study site in Radcliffe on Trent in Nottinghamshire, UK (hybrid satellite image and map taken from Google Earth). The four fields labelled in yellow were home to one herd of cattle (n = 23 adult female), while the three fields labelled in blue were home to the other herd (n = 21 adult female).

The two groups of fields were approximately of 52 Ha (Herd 1) and 23 Ha (Herd 2) in area. I recorded 344 calls (cows, n = 205; calves, n = 139, see Tables 2.1, 2.2 & 2.3 for details) from 31 individuals (cows, n = 17; calves, n = 14). All individuals included in the study were free to roam in the fields with fresh grass and water *ad libitum*. The calves included were all born between February and July 2010, and were all sired by the same bull.

Table 2.1. Individual cow HFC's recorded during the field work season 2010

Individual	February	March	April	May	June	July	August	Total
Alfalfa		1	6		2			**9**
Black cheek		9	1					**10**
Black udders		5				2	1	**8**
Blue		1	7					**8**
Cecil			8	1				**9**
Cinnamon			9					**9**
Dark face		1	9					**10**
Evil			10					**10**
Freckles		3	7					**10**
Grey			5	1		4		**10**
Stine						7		**7**
T nose	5	2	1					**8**
Tikva			6	1			1	**8**
Up & Down			8			2		**10**
White udders			3	7				**10**
Total calls per individual	**5**	**22**	**80**	**10**	**2**	**15**	**2**	**136**

Table 2.2. Individual cow LFC's recorded during the field work season 2010

Individual	February	March	April	May	June	July	August	Total
Alfalfa		7	1					**8**
Black tips		10						**10**
Cinnamon			7					**7**
Evil		9	1					**10**
Helena		10						**10**
T nose		9						**9**
Tikva			7	1				**8**
Up & Down		6				1		**7**
Total calls per individual	**0**	**51**	**16**	**1**	**0**	**1**	**0**	**69**

Table 2.3. Individual calf recordings during the field work season 2010

Individual	February	March	April	May	June	July	August	Total
407			3	2	1	1	2	**9**
411				7		3		**10**
Ali		2	4			2	2	**10**
Ashes		1	2	1		3	3	**10**
Athena		7	1			2		**10**
Brad			4	3	3			**10**
Frantz						7	3	**10**
Ginger				4	2		4	**10**
Meredith		5	5					**10**
Milky			5	5				**10**
Piojillo		4		1		2	3	**10**
Rojilla		10						**10**
Silvia				6		1	3	**10**
Tikvo		1	3	3		2	1	**10**
Total calls per individual		**30**	**27**	**32**	**6**	**23**	**21**	**139**

The two herds were kept separately in their fields almost without interchange of animals, except in three occasions, where the owners considered that some individuals should be swapped between fields, to match cows with one of two bulls according to size. Additionally, there were two occasions when two cows were isolated for medical treatments for up to two weeks. All the calves included in the study were kept all year long in the same field with their mothers.

2.3.2 Sound recording and signal acquisition

Recordings of individual calves and cows were made opportunistically between 8 am and 5 pm, from February 2010 to December 2010. Calls were recorded at distances of 10 – 30 m from the vocalizing animal with a Sennheiser MKH70 directional microphone, connected to a Marantz PMD660 digital recorder (sampling rate 44.1 kHz). Accurate, individual identification was done from specific ID tags placed in the animals' ears by the farmers, and by visual recognition of coat markings. Because of the farmer's records, the exact ages of the calves at the moment that calls were recorded were known. Unfortunately, a shortage of appropriate records meant that the age of the cows was not known, but all were at least two years old.

Vocalizations were uploaded to a computer at a sampling rate of 44.1 kHz and saved in a WAV format at 16-bit amplitude resolution. I used Praat v.5.1.44 DSP

Package (Boersma and Weenink 2009) for the acoustic analysis. Calls were individually visualized in spectrograms in Praat (FFT method, window length = 0.1 s, time steps = 100, frequency steps = 250, Gaussian window shape, dynamic range = 40 dB). Vocalizations with high levels of background noise (as visualized in the spectrogram) were not considered for acoustic analysis.

2.3.3 Acoustic analyses

Calf and cow vocalizations were typically 1.3 – 1.5 seconds long, with a clear harmonic structure (Figs 2.2 – 2.4). Cow calls were divided in two different basic categories. Low frequency calls (henceforth "LFCs") were made with the mouth closed or only partially opened. They were extremely quiet, being noticeable to a casual observer only when produced indoors away from background noise typically encountered in the field (Fig. 2.2). By contrast, high frequency calls (henceforth "HFCs"), where the cow's mouth was fully opened for at least part of the call (the call sometimes started with the mouth only partially opened), were typically much louder, and were clearly audible in the field (Fig. 2.3).

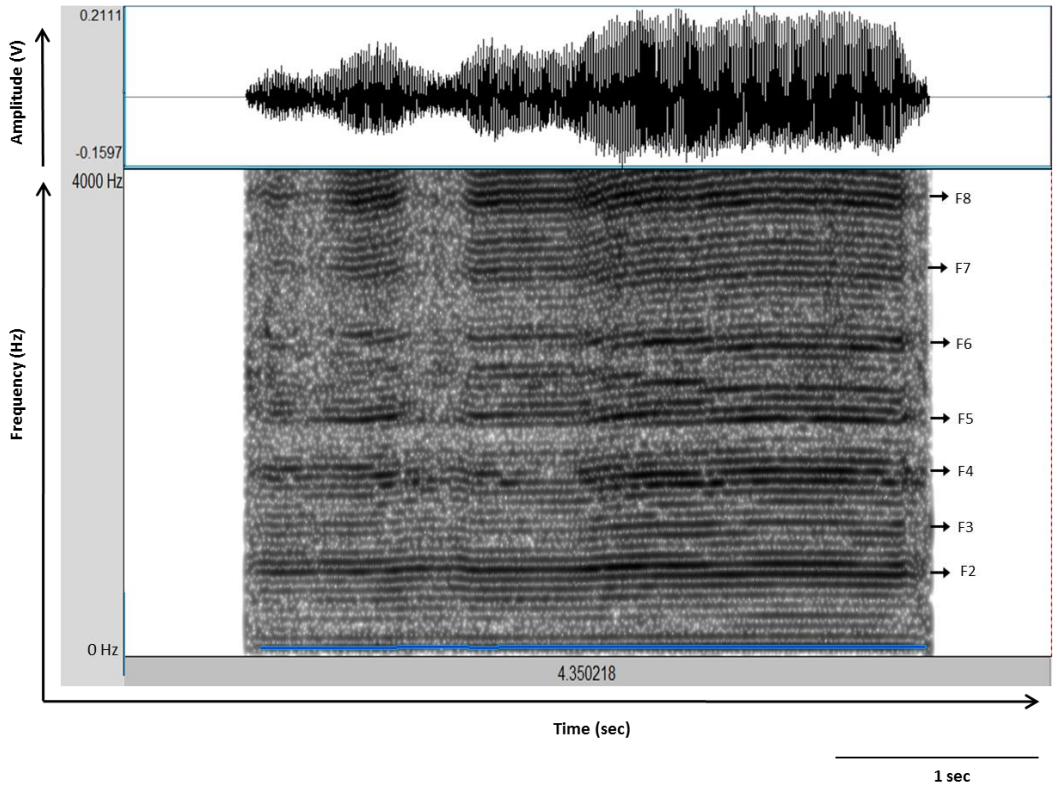

Figure 2.2 Waveform (above) and spectrogram (below) of one low frequency cow call (LFC). The blue line at the bottom indicates the fundamental frequency (F0). The arrows indicate the frequency values of the seven formants (F2 - F8).

LFCs were in all cases observed after calving, and when a cow was close to its offspring. HFCs were more typically: a) Contact calls, from cows that were looking for their calves while these were out of sight, b) Moving away calls, when the whole herd was moving to a different field, or c) Alarm calls, when there was an unusual situation in the field, such as the presence of machinery or unknown people with dogs. For the purposes of this study, I have considered only contact calls produced while mothers were looking for their calves or vice versa.

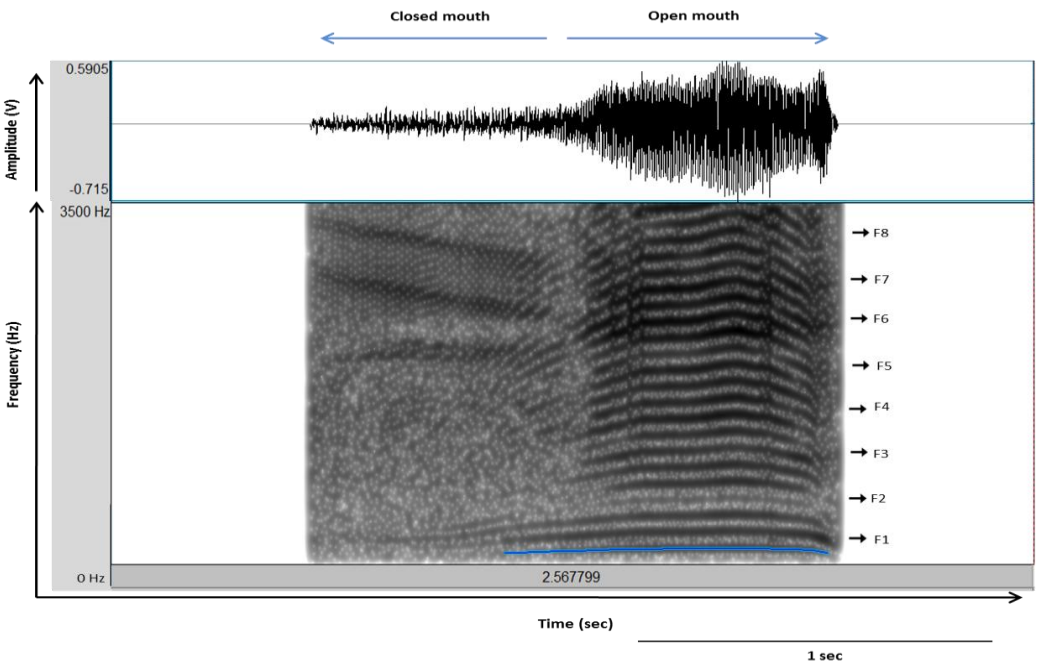

Figure 2.3 Waveform (above) and spectrogram (below) of one high frequency cow call (HFC). The blue line at the bottom indicates the fundamental frequency (F0). The arrows indicate the frequency values of the eight formants (F1 - F8). In this case the first part of the call was produced with the mouth partially closed, and then the second part with the mouth fully open (the arrows at the top indicate where the change occurs). Therefore, the two parts have different acoustic parameters. I considered for HFCs just the part with open mouth and the acoustic parameters described in this figure correspond exclusively to the acoustic analysis carried out on the part of the call made with opened mouth.

Calf calls were typically more high-pitched vocalizations, made with the mouth fully opened for at least some of the time (Fig 2.4). As with cow HFCs, the first part of the call was sometimes (c. 30 % of calls) made with the mouth only partially opened.

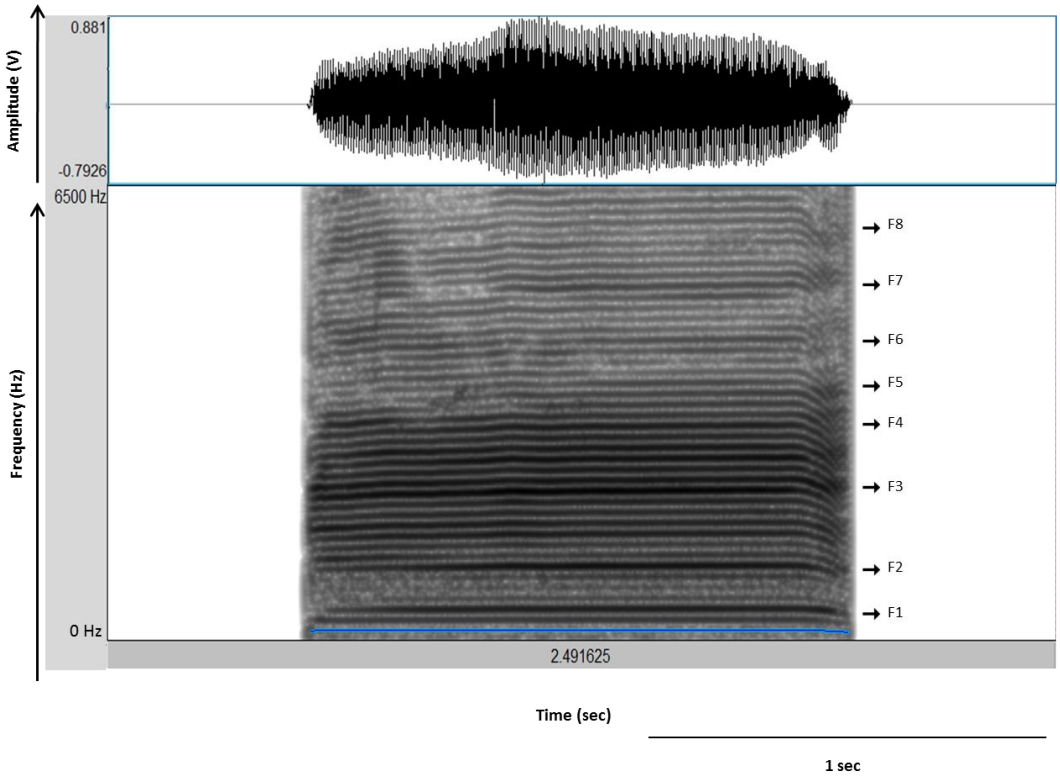

Figure 2.4 Waveform (above) and spectrogram (below) of a single calf call. The blue line at the bottom indicates the fundamental frequency (F0). The arrows indicate the frequency values of the eight formants (F1 - F8).

In order to assess the basic acoustic parameters of cattle calls, the source-related vocal features were extracted (parameters related to the fundamental frequency, F0), together with filter-related features (formants) and intensity measures (45 parameters in total for cow and calf calls; Table 2.4), all of which potentially contribute to vocal distinctiveness (Taylor and Reby 2010), using a custom built programme in Praat v.5.1.44 (Reby and McComb 2003). This programme batch-processed the calls, editing them, running analyses and exporting data, except for the filter-related features, which were manually and individually calculated in Praat. For calls which were composed of two parts, the first made with the mouth fully or partially closed, I recorded the original total call length but, in order to accurately calculate the acoustic parameters, I analysed just the part of the call made with the mouth open.

Table 2.4. The vocal parameters analysed in calf and cow calls, with the abbreviations used throughout the thesis.

Abbreviation	Parameter
F0Start (Hz)	Frequency value of F0at the start of the call
F0End (Hz)	Frequency value of F0 at the end of the call
F0Mean (Hz)	Mean F0 frequency value across the call
F0Min (Hz)	Minimum F0 frequency value across the call
F0Max (Hz)	Maximum F0 frequency value across the call
Time F0Max (%)	Percentage of the total call duration when F0 is maximum
F0AbsSlope (Hz/s)	F0 mean absolute slope
F0Var (Hz/s)	Cumulative variation in the F0 contour in Hertz divided by call duration
FMRate (s-1)	Number of complete cycles of F0 modulation per second
FMExtent (Hz)	Mean peak-to-peak variation of each F0 modulation
Jitter (%)	Mean absolute difference between frequencies of consecutive F0 periods divided by the mean frequency of F0
Shimmer (%)	Mean absolute difference between the amplitudes of consecutive F0 periods divided by the mean amplitude of F0
F1Mean (Hz)	Mean frequency value of the first formant
F2Mean (Hz)	Mean frequency value of the second formant
F3Mean (Hz)	Mean frequency value of the third formant
F4Mean (Hz)	Mean frequency value of the fourth formant
F5Mean (Hz)	Mean frequency value of the fifth formant
F6Mean (Hz)	Mean frequency value of the sixth formant
F7Mean (Hz)	Mean frequency value of the seventh formant
F8Mean (Hz)	Mean frequency value of the eighth formant
F1Min (Hz)	Minimum frequency value of the first formant
F2Min (Hz)	Minimum frequency value of the second formant
F3Min (Hz)	Minimum frequency value of the third formant
F4Min (Hz)	Minimum frequency value of the fourth formant
F5Min (Hz)	Minimum frequency value of the fifth formant
F6Min (Hz)	Minimum frequency value of the sixth formant
F7Min (Hz)	Minimum frequency value of the seventh formant
F8Min (Hz)	Minimum frequency value of the eighth formant
F1Max (Hz)	Maximum frequency value of the first formant
F2Max (Hz)	Maximum frequency value of the second formant
F3Max (Hz)	Maximum frequency value of the third formant
F4Max (Hz)	Maximum frequency value of the fourth formant
F5Max (Hz)	Maximum frequency value of the fifth formant
F6Max (Hz)	Maximum frequency value of the sixth formant
F7Max (Hz)	Maximum frequency value of the seventh formant
F8Max (Hz)	Maximum frequency value of the eighth formant
Df Min (Hz)	Minimum spacing of the formants
Max VTL (s)	Estimated vocal tract length
Q25% (Hz)	Frequency value at the upper limit of the first quartiles of energy
Q50% (Hz)	Frequency value at the upper limit of the second quartiles of energy
Q75% (Hz)	Frequency value at the upper limit of the third quartiles of energy
Amp Var (dB/s)	Cumulative variation in amplitude divided by the total call duration
AMRate (s-1)	Number of complete cycles of amplitude modulation per second
AMExtent (dB)	Mean peak-to-peak variation of each amplitude modulation
Dur (s)	Duration of the call

2.3.4 Statistical analysis

Cow calls were divided in two different groups, and sample sizes were as follows: for HFCs, n = 15 individuals, with 7 - 10 calls per individual; for LFCs, n = 8 individuals, with 7-10 calls per individual; for calf calls, n = 14 individuals, with 9 - 10 calls per individual. For individuals for which there were more calls than this available, and in order to create a balanced design for the analysis, I discarded a random selection of calls.

Individual distinctiveness of calls was calculated for cow and calf contact calls by calculating the Potential of Individual Coding (PIC) for each measured parameter, and by performing a Principal Components Analysis (PCA), followed by a Multivariate Analysis of Variance (MANOVA) and a Discriminant Function Analysis (DFA). Calls of calves and cows were treated separately. HFCs and LFCs were also treated separately.

To calculate PICs, coefficients of variation were first calculated between and within individuals (CVb and CVw, respectively) as follows:

$$CV = 100\left(1 + \frac{1}{4n}\right)\left(\frac{SD}{\bar{x}}\right)$$

where *SD* is the standard deviation, $\bar{x}$ is the mean of the sample and *n* is the sample size (Sokal and Rohlf 1995).

PIC was then calculated as the ratio of CVb to the mean CVw for all individuals. For a given parameter, a PIC value greater than 1 indicates that this parameter has good potential for use in individual recognition because its intra-individual variability is smaller than in inter-individual variability (Robisson et al. 1993).

PCA was used to eliminate redundancy due to the high intercorrelation of the measured vocal parameters, and to examine clustering among parameters. Missing data, occurring when one vocal parameter in a given call could not be measured, were replaced by the average value of this parameter for the given individual (0.5 % of values missing for calves, 1.0 % HFCs and 0.06 % LHCs). The principal components (PCs) with Eigen values of greater than 1 were retained, and were then used as input variables for the subsequent statistical analysis (as in Briefer and McElligott 2011).

MANOVA with "individual" as fixed factor and "age" (in calves) included as a covariate was performed in order to confirm statistical differences among individuals in PC scores. Then DFA with one factor (individual) was used to

quantify the extent to which individuals can be classified on the basis of their calls, and to indentify which groups of variables (PCs) are most useful in this classification. On the basis of the discriminant functions from the DFA, each set of PC scores (corresponding to a call) was assigned to the appropriate individual (correct classification) or to another individual (incorrect classification). This allowed me to calculate the percentage of calls correctly classified (CC). The results were cross-validated by performing a leave one out classification (McGarigal et al. 2000). I then calculated the CC due to the chance by applying a randomisation procedure. The expected level of correct assignment was averaged from DFAs performed on 1,000 randomised permutations of the data set (McGarigal et al. 2000).

Conventional DFA only allows the inclusion of a single factor at a time, and differences among individual calves identified in my initial DFA could arise solely because of differences between males and females. To remove the potentially confounding effect of sex, two additional DFAs were carried out on male and female data separately. The CCs were calculated for these DFAs as previously described.

Some additional analysis was performed to check for effects of sex on vocalisations in calves. MANOVA with “sex” as fixed factor (in calves), was

performed on the average PC scores per individual in order to test for statistical differences between female and male calls.

In order to determine whether the age-related changes in calf vocalizations are different in males and females, I performed a T-test on the slopes of the relationship between age and the first two PCs for each calf.

2.4 Results

PIC values and all mean values of the vocal parameters obtained for calves and cows (HFC and LFC) are shown in Table 2.5. For cow LFCs, the amplitude of the first formant was low compared to the subsequent formants and the software could not track it accurately; therefore it was eliminated from the analysis. PICs for most vocal parameters analysed in calf and cow calls (both LFCs and HFCs) were greater than 1, except for Time F0Max in calves, and FMRate in cow LFCs. This indicates that most of the filter- and source-related parameters are likely to code for individuality in calf and cow calls.

PCA generated nine PCs for both cow LFCs (n = 69 calls, 8 cows and 42 vocal parameters) and calf calls (n = 139 calls, 14 calves and 45 vocal parameters), and in both cases these PCs explained over 84 % of the variance in the original variables used to describe calls (Tables 2.6 and 2.7). In both cases, the first two PCs together explained more than half of the variance in the original variables. The first PC was strongly and positively correlated with all the parameters describing the frequencies of the formants, and the minimum spacing among formant frequencies, but negatively with the mean vocal tract length (VTL). The second PC correlated strongly and positively with the parameters describing the fundamental frequency.

The results of the PCA for cow HFCs were slightly different. Twelve PCs were generated (n = 136 calls, 15 cows and 45 vocal parameters), which together explained 76 % of the variance in the original variables used to describe the calls (Table 2.8). The first three PCs each explained more than 9 % of the original variance. As with the calf calls and cow LFCs, the first PC correlated strongly and positively with the mean and maximum formant frequencies, but it did not correlate with the minimum formant frequencies or the VTL. The second PC correlatedOwn positively with several attributes of the fundamental frequency contour, and it also correlated negatively with the VTL and some other parameters. The third PC was more like the second PC for calves and cow LFCs, in that it correlated positively with the fundamental frequency, although it also correlated with some formant frequencies.

Table 2.5. Mean vocal parameters and PIC values (mean ± SEM) for high and low frequency cow calls (HFCs and LFCs), and calf calls. See Table 2.1 for abbreviations of the vocal parameters.

Parameter	LFC Cows			HFC Cows			Calves		
	Mean	SEM	PIC	Mean	SEM	PIC	Mean	SEM	PIC
F0 Start (Hz)	76.52	0.96	1.44	94.24	3.24	1.33	130.1	2.11	1.16
F0 End (Hz)	80.08	1.15	1.56	145.4	3.61	1.26	129.8	2.15	1.16
F0 Mean (Hz)	81.17	0.980	1.47	152.8	3.10	1.27	142.8	1.8	1.22
F0 Mix (Hz)	74.84	1.01	1.40	91.05	2.87	1.31	121.0	1.68	1.28
F0 Max (Hz)	84.76	1.04	1.51	198.7	3.62	1.45	153.3	2.18	1.12
Time Max F0	66.78	2.75	1.07	73.64	1.51	1.13	65.42	1.72	0.98
F0Abs Slope	18.85	1.61	1.32	150.9	6.75	1.23	55.77	3.29	1.12
F0 Var (Hz/s)	12.97	1.34	1.25	132.4	6.08	1.20	35.94	2.11	1.00
Fm Rate (s-1)	1.78	0.14	0.97	2.15	0.11	1.03	1.26	0.07	1.07
FM Extend (Hz)	10.5	1.35	1.15	96.78	7.7	1.10	45.80	4.00	1.21
Jitter (%)	0.02	0.00	1.23	0.04	0.00	1.08	0.01	0.00	1.34
Shimmer (%)	0.17	0.00	1.19	0.17	0.00	1.09	0.15	0.00	1.02
F1 Mean (Hz)				228.3	1.85	1.09	391.7	5.37	1.12
F2 Mean (Hz)	634.3	6.66	1.24	644.6	3.79	1.19	1162	16.09	1.12
F3 Mean (Hz)	1064	11.77	1.14	1073	2.84	1.19	1939	24.66	1.12
F4Mean (Hz)	1513	16.19	1.21	1478	2.59	1.12	2722	34.27	1.16
F5 Mean (Hz)	1930	20.11	1.22	1889	2.48	1.12	3499	42.39	1.16
F6 Mean (Hz)	2384	23.04	1.14	2319	2.46	1.03	4280	50.37	1.17
F7 Mean (HZ)	2819	25.20	1.17	2743	2.29	1.14	5050	60.49	1.14
F8 Mean (Hz)	3224	26.28	1.24	3181	2.69	1.10	5813	68.79	1.15
F1 Min (Hz)				171.8	2.35	1.09	312.9	5.83	1.05
F2 Min (Hz)	543.9	8.73	1.23	552.1	4.20	1.12	1018	15.62	1.09
F3 Min (Hz)	961.2	12.64	1.13	971.3	3.88	1.10	1782	24.11	1.10
F4 Min (Hz)	1403	17.05	1.24	1381	3.12	1.05	2561	33.82	1.16
F5 Min (Hz)	1814	20.50	1.24	1788	2.93	1.08	3335	42.43	1.13
F6 Min (Hz)	2273	23.74	1.13	2210	3.15	1.04	4108	49.59	1.17
F7 Min (Hz)	2697	26.10	1.21	2630	2.92	1.05	4860	59.66	1.13
F8 Min (Hz)	3099	27.59	1.19	3062	2.97	1.00	5627	68.39	1.16
F1 Max (Hz)				301.3	3.64	1.06	465.5	7.31	1.18
F2 Max (Hz)	735.8	8.24	1.21	745.0	4.87	1.15	1311	17.33	1.12
F3 Max (Hz)	1186	12.16	1.08	1174	4.11	1.03	2089	25.97	1.14
F4 Max (Hz)	1631	17.47	1.17	1587	4.41	1.01	2891	35.22	1.16
F5 Max (Hz)	2045	20.22	1.20	2005	4.16	1.00	3682	42.78	1.18
F6 Max (Hz)	2494	23.58	1.14	2446	4.43	1.03	4467	51.36	1.19
F7 Max (Hz)	2943	25.38	1.14	2870	4.44	1.06	5248	61.51	1.17
F8 Max (Hz)	3365	26.21	1.21	3313	4.84	1.03	6022	69.36	1.16
Df Min (Hz)	426.3	3.78	1.13	413.5	0.57	1.16	763.5	9.14	1.17
Max VTL (cm)	41.26	0.35	1.10	42.32	0.05	1.16	23.40	0.29	1.20
Q25% (Hz)	112.6	5.08	1.10	172.6	4.44	1.14	259.8	14.16	1.12
Q50% (Hz)	353.1	23.36	1.14	290.9	10.39	1.24	543.2	26.01	1.10
Q75% (Hz)	1227	92.62	1.05	595.7	32.19	1.29	1103	53.72	1.01
Amp Var (dB/s)	8.67	0.38	1.15	38.22	1.16	1.07	11.06	0.44	1.04
AM Rate (s-1)	2.32	0.07	1.01	9.56	0.21	1.04	2.95	0.09	1.01
AM Extent (dB)	4.10	0.25	1.06	4.34	0.25	1.71	4.55	0.29	1.30
Duration (s)	1.30	0.06	1.25	1.29	0.04	1.28	1.44	0.06	1.15

Table 2.6. Results of Principal Component Analysis (PCA) of vocal parameters from calf calls. Bold type indicate loadings > 0.5.

	Component								
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	24.62	4.11	3.32	2.73	2.00	1.80	1.51	1.25	1.03
Variance explained	49.25	8.23	6.65	5.47	4.00	3.61	3.03	2.50	2.06
Loadings:									
F0 Start (Hz)	0.129	**0.840**	-0.084	-0.080	-0.098	-0.231	0.258	-0.091	-0.032
F0 End (Hz)	0.058	**0.818**	0.037	0.009	-0.037	0.096	-0.237	0.143	0.104
F0 Mean (Hz)	-0.007	**0.911**	-0.084	0.113	-0.073	-0.001	0.126	0.029	-0.084
F0 Min (Hz)	0.131	**0.871**	-0.137	-0.165	-0.251	-0.051	-0.067	0.011	-0.034
F0 Max (Hz)	-0.080	**0.896**	0.029	0.297	0.108	-0.012	0.076	0.034	-0.065
Time F0 Max (%)	-0.117	0.150	0.152	0.213	-0.001	0.436	**-0.526**	0.302	0.168
F0 Abs Slope	-0.035	0.174	0.237	0.439	**0.673**	-0.090	0.155	-0.137	0.269
F0 Var (HZ/s)	-0.186	0.186	0.370	0.**527**	**0.506**	-0.050	-0.047	-0.295	0.172
Fm Rate (s-1)	0.186	0.173	-0.050	-0.417	-0.044	-0.152	-0.009	**-0.523**	0.487
FM extend (Hz)	-0.269	0.021	0.238	**0.616**	0.287	0.041	0.043	0.103	-0.395
Jitter (%)	0.206	0.193	-0.298	-0.146	0.120	-0.204	-0.115	0.235	0.182
Shimmer (%)	0.107	0.026	**-0.759**	0.017	0.203	-0.122	0.007	-0.213	-0.157
F1(mean) (Hz)	**0.892**	0.047	-0.026	0.022	-0.093	-0.012	0.056	-0.182	-0.033
F2(mean) (Hz)	**0.843**	0.012	0.083	-0.026	0.011	0.190	0.049	0.193	0.254
F3(mean) (Hz)	**0.967**	0.004	0.045	0.040	0.041	0.017	0.022	-0.017	0.010
F4(mean) (Hz)	**0.983**	0.000	0.047	0.039	-0.008	-0.004	0.001	0.012	-0.012
F5(mean)(Hz)	**0.984**	-0.005	0.023	0.010	-0.032	0.025	-0.016	-0.030	-0.055
F6(mean)(Hz)	**0.990**	-0.005	0.005	0.011	-0.001	0.014	-0.009	-0.006	-0.003
F7(mean)(Hz)	**0.992**	-0.001	0.023	0.029	-0.002	-0.001	0.009	0.001	-0.028
F8(mean)(Hz)	**0.988**	-0.011	0.016	0.046	-0.016	0.022	-0.023	-0.009	-0.028
F1(min)(Hz)	**0.694**	-0.043	-0.080	0.074	-0.205	0.105	-0.155	-0.285	-0.154
F2(min)(Hz)	**0.830**	0.036	0.179	-0.059	0.005	0.197	0.026	0.164	0.235
F3(min)(Hz)	**0.946**	-0.019	0.082	0.028	0.056	0.024	0.010	0.007	0.024
F4(min)(Hz)	**0.979**	0.018	0.058	0.063	0.011	-0.002	-0.002	0.018	-0.005
F5(min)(Hz)	**0.978**	0.001	0.042	0.006	-0.029	0.038	-0.016	-0.029	-0.061
F6(min)(Hz)	**0.984**	-0.012	0.015	0.034	0.005	0.025	-0.015	-0.016	-0.031
F7(min)(Hz)	**0.987**	0.001	0.027	0.033	-0.014	0.000	-0.007	-0.005	-0.041
F8(min)(Hz)	**0.982**	-0.011	0.015	0.057	-0.027	0.018	-0.032	-0.020	-0.052
F1(max)(Hz)	**0.795**	0.088	-0.056	-0.013	0.007	-0.048	0.111	-0.109	-0.076
F2(max)(Hz)	**0.812**	-0.007	-0.032	0.001	0.104	0.180	0.022	0.229	0.267
F3(max)(Hz)	**0.958**	0.015	0.000	0.067	0.036	0.030	0.019	0.003	-0.015
F4(max)(Hz)	**0.973**	-0.006	0.021	0.027	-0.001	0.027	0.005	0.032	-0.008
F5(max)(Hz)	**0.977**	-0.018	0.017	-0.014	-0.036	0.037	-0.021	-0.018	-0.055
F6(max)(Hz)	**0.984**	-0.004	-0.014	-0.005	-0.012	0.021	-0.007	0.005	-0.011
F7(max)(Hz)	**0.989**	-0.004	0.019	0.025	0.001	0.018	0.003	0.012	-0.033
F8(max)(Hz)	**0.986**	-0.010	-0.002	0.039	-0.004	0.043	-0.020	0.008	-0.023
Df(min)(Hz)	**0.980**	-0.010	-0.014	0.055	-0.006	-0.010	-0.024	-0.024	-0.072
Max VTL (cm)	**-0.976**	0.033	0.029	-0.043	-0.010	0.041	0.003	0.017	0.081
Q25% (Hz)	-0.192	-0.018	**0.784**	0.145	-0.255	-0.016	0.003	-0.189	-0.069
Q50% (Hz)	0.030	0.004	**0.865**	-0.193	-0.092	**-0.060**	0.167	-0.001	-0.114
Q75% (Hz)	0.108	0.064	**0.700**	-0.281	0.116	-0.163	0.203	0.270	0.013
AM Var (dB/s)	0.251	-0.133	-0.396	-0.031	0.468	-0.349	-0.178	0.280	-0.081
AM rate (s-1)	0.021	-0.032	-0.300	-0.248	0.453	**0.549**	0.412	0.056	-0.026
AM extent (dB)	0.175	-0.048	0.062	0.288	-0.046	**-0.678**	-0.472	0.183	-0.018
Duration (s)	-0.462	0.173	-0.104	0.038	-0.359	0.219	0.094	0.268	-0.047

Table 2.7. Results of Principal Component Analysis (PCA) vocal parameters from low frequency cow calls (LFCs). Bold type indicates loadings > 0.5

	Component								
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	19.20	5.99	4.17	2.82	1.91	1.80	1.54	1.26	1.10
Variance explained %	41.74	13.03	9.07	6.13	4.16	3.92	3.354	2.74	2.39
Loadings:									
F0 Start	-0.143	**0.754**	0.489	0.134	0.208	-0.027	0.020	0.051	0.054
F0 End (Hz)	-0.174	0.820	0.389	0.175	0.068	-0.117	-0.047	0.032	-0.017
F0 Mean (Hz)	-0.130	0.831	0.389	0.310	-0.007	-0.072	0.041	-0.050	0.040
F0 Min (Hz)	-0.150	0.822	0.473	0.057	0.074	0.061	0.011	0.069	-0.020
F0 Max F0	-0.153	**0.754**	0.315	0.446	0.091	-0.254	0.020	-0.031	0.093
Time F0 Max (%)	-0.017	**0.506**	-0.022	-0.140	0.384	-0.154	-0.041	0.224	-0.303
F0 Abs Slope (Hz/s)	-0.100	**-0.523**	-0.118	**0.533**	0.391	-0.451	-0.045	0.032	0.040
F0 Var (Hz/s)	-0.052	-0.425	-0.176	**0.582**	0.401	-0.476	-0.091	0.062	-0.012
Fm Rate (s-1)	-0.014	-0.272	0.207	-0.014	0.695	0.003	-0.076	**0.504**	-0.132
FM extend (Hz)	0.120	-0.097	-0.289	**0.613**	-0.204	-0.148	-0.009	**-0.539**	0.217
Jitter (%)	-0.105	**-0.518**	0.308	0.319	-0.011	0.060	0.406	0.044	-0.292
Shimmer (%)	-0.206	-0.428	0.468	0.390	-0.008	0.222	-0.070	0.080	0.075
F2 (mean) (Hz)	**0.835**	-0.135	0.016	0.106	0.152	0.264	-0.136	-0.086	-0.121
F3 (mean) (Hz)	**0.848**	-0.054	0.085	0.193	0.048	0.157	-0.166	-0.035	0.117
F4 (mean) (Hz)	**0.955**	0.090	0.000	-0.043	0.060	-0.019	-0.003	0.031	-0.008
F5 (mean) (Hz)	**0.952**	-0.029	0.070	-0.005	-0.024	-0.013	0.002	-0.039	-0.064
F6 (mean) (Hz)	**0.963**	0.035	0.083	-0.013	-0.098	0.035	0.018	-0.038	0.045
F7 (mean) (Hz)	**0.961**	0.072	0.056	0.002	-0.032	-0.066	0.075	0.042	0.072
F8 (mean) (Hz)	**0.968**	0.001	-0.007	-0.029	0.012	-0.095	0.092	0.064	-0.036
F2 (min) (Hz)	**0.669**	0.002	-0.001	0.011	0.272	0.402	-0.271	-0.078	-0.164
F3 (min) (Hz)	**0.830**	0.023	0.064	0.146	0.008	0.106	-0.117	-0.016	0.101
F4 (min) (Hz)	**0.914**	0.159	0.009	-0.055	0.023	-0.002	-0.022	-0.016	-0.054
F5 (min) (Hz)	**0.942**	-0.035	0.020	-0.036	-0.050	-0.031	0.001	-0.022	-0.079
F6 (min) (Hz)	**0.949**	0.038	0.051	-0.061	-0.050	0.085	0.036	0.033	0.063
F7 (min) (Hz)	**0.964**	0.098	0.028	-0.046	0.001	-0.025	0.068	0.075	0.040
F8 (min) (Hz)	**0.960**	0.006	0.008	0.012	-0.015	-0.092	0.090	0.098	-0.079
F2 (max) (Hz)	**0.692**	-0.274	0.091	0.164	-0.033	0.086	0.022	-0.174	-0.170
F3 (max) (Hz)	**0.781**	-0.201	0.021	0.213	0.036	0.145	-0.231	-0.059	0.149
F4 (max) (Hz)	**0.930**	-0.018	0.008	-0.012	-0.002	-0.077	-0.045	0.005	0.053
F5 (max) (Hz)	**0.933**	-0.045	0.078	0.016	-0.015	-0.037	0.004	-0.033	-0.065
F6 (max) (Hz)	**0.943**	0.040	0.144	0.080	-0.107	0.002	-0.003	-0.035	0.048
F7 (max) (Hz)	**0.937**	0.060	0.067	0.030	-0.040	-0.134	0.068	0.039	0.072
F8 (max) (Hz)	**0.937**	0.050	0.024	-0.023	0.013	-0.114	0.125	0.058	-0.040
Df(min) (Hz)	**0.927**	0.068	0.018	-0.026	-0.053	-0.156	0.164	0.167	0.015
Max VTL (cm)	**-0.915**	-0.620	-0.030	0.009	0.054	0.165	-0.171	-0.171	0.000
Q25% (Hz)	0.057	0.429	**-0.521**	0.291	0.211	0.398	0.238	-0.133	0.172
Q50% (Hz)	0.074	0.280	**-0.718**	0.155	0.188	0.198	0.471	0.096	0.084
Q75% (Hz)	0.041	0.135	**-0.696**	0.053	0.143	0.129	**0.561**	0.155	0.107
AM Var (dB/s)	0.068	-0.328	**0.601**	-0.183	-0.012	-0.147	0.395	-0.235	0.032
AM rate (s-1)	0.024	-0.131	-0.220	**0.551**	**-0.543**	0.007	-0.131	0.285	0.032
AM extent (dB)	0.000	-0.193	**0.557**	-0.419	0.328	-0.110	0.352	-0.401	0.060
Duration (s)	0.002	0.302	-0.094	-0.146	-0.191	**-0.506**	0.073	0.093	0.202

Table 2.8. Results of Principal Component Analysis (PCA) vocal parameters from high frequency cow calls (HFCs). Bold type indicates loadings > 0.5.

	Component											
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Eigenvalue	8.45	7.26	4.83	3.21	2.72	2.18	1.87	1.70	1.50	1.25	1.21	1.11
Variance explained %	17.24	14.82	9.87	6.56	5.56	4.45	3.83	3.48	3.07	2.56	2.48	2.27
Loadings:												
F0 start (Hz)	-0.410	0.240	0.541	-0.062	**-0.551**	0.119	-0.073	-0.080	0.107	-0.007	-0.134	0.056
F0 end (Hz)	-0.087	0.415	0.347	0.076	0.074	0.200	0.085	-0.252	0.492	0.217	0.297	0.087
F0 Mean (Hz)	-0.401	0.488	**0.590**	-0.013	-0.155	-0.026	-0.127	-0.097	0.114	0.100	0.230	0.063
F0 Min (Hz)	-0.038	0.252	**0.551**	-0.078	**-0.527**	0.186	-0.101	-0.084	0.129	0.009	-0.090	0.064
F0 Max (Hz)	-0.098	**0.574**	0.453	0.289	0.233	-0.138	0.150	-0.119	0.283	0.135	0.259	0.053
Time Max F0 (%)	0.474	-0.093	-0.491	-0.136	0.229	0.026	-0.022	0.021	0.406	0.088	0.066	-0.025
F0 abs slope (Hz/s)	-0.139	**0.575**	0.367	0.257	0.492	-0.208	-0.096	-0.023	-0.024	0.002	0.088	-0.011
F0 Var (HZ/s)	-0.160	**0.517**	0.380	0.247	**0.508**	-0.252	-0.082	-0.062	-0.224	-0.017	0.175	-0.022
Fm Rate (s-1)	-0.161	0.058	-0.044	0.495	-0.425	0.175	0.353	-0.247	-0.133	0.064	0.263	-0.045
FM extend (Hz)	0.046	0.256	0.234	-0.249	**0.655**	-0.271	-0.292	0.096	0.087	-0.008	-0.133	0.037
Jitter (%)	0.109	0.455	-0.144	**0.672**	0.060	-0.090	-0.008	0.033	-0.108	0.187	-0.033	-0.015
Shimmer (%)	0.249	0.405	-0.284	**0.558**	-0.070	-0.119	-0.210	0.094	-0.110	0.079	-0.013	0.117
F1 (mean) (Hz)	0.169	**-0.516**	0.154	0.095	0.277	0.146	0.225	-0.018	0.011	-0.475	0.034	0.278
F2 (mean) (Hz)	**0.594**	-0.429	0.188	0.217	-0.120	0.244	-0.182	0.235	-0.001	0.148	0.145	0.032
F3 (mean) (Hz)	**0.606**	-0.318	0.453	0.012	-0.158	-0.285	-0.179	0.020	-0.031	0.028	0.096	-0.016
F4 (mean) (Hz)	**0.639**	0.037	**0.572**	-0.015	-0.008	-0.119	0.127	0.018	0.101	0.125	-0.083	0.112
F5 (mean) (Hz)	**0.700**	0.178	0.311	-0.021	-0.052	-0.140	0.255	-0.088	0.071	-0.106	-0.130	-0.276
F6 (mean) (Hz)	**0.703**	0.200	0.251	-0.127	-0.095	-0.152	0.135	0.096	-0.096	-0.200	0.002	-0.105
F7 (mean) (Hz)	**0.717**	0.403	0.054	0.035	-0.049	0.143	0.128	0.046	-0.031	-0.122	0.140	-0.257
F8 (mean) (Hz)	**0.596**	0.415	-0.100	-0.114	-0.116	0.289	-0.073	0.090	-0.246	-0.063	0.162	-0.018
F1 (min) (Hz)	-0.303	-0.415	0.307	0.058	0.155	-0.049	0.218	-0.073	-0.009	-0.401	0.261	-0.024
F2 (min) (Hz)	0.295	**-0.535**	0.270	0.255	-0.112	0.210	0.051	0.207	-0.168	0.151	0.171	-0.116
F3 (min) (Hz)	0.362	-0.330	0.313	0.129	-0.144	-0.326	-0.316	0.103	-0.018	-0.057	0.142	-0.099
F4 (min) (Hz)	0.264	-0.018	0.410	-0.146	0.013	-0.231	0.298	0.233	-0.041	0.299	-0.030	0.081
F5 (min) (Hz)	0.396	0.092	0.301	0.001	-0.049	-0.158	0.409	0.236	0.197	0.173	-0.197	-0.284
F6 (min) (Hz)	0.140	0.203	0.416	-0.332	-0.169	-0.130	0.064	0.235	-0.244	-0.179	0.100	0.018
F7 (min) (Hz)	0.313	0.424	-0.074	-0.030	-0.024	0.073	0.268	0.194	-0.183	-0.092	0.305	-0.014
F8 (min) (Hz)	0.175	0.375	-0.093	-0.312	0.012	0.158	0.228	0.404	-0.138	0.153	0.180	0.265
F1 (max) (Hz)	0.470	-0.374	0.005	0.103	0.105	0.103	0.132	-0.146	-0.062	-0.090	-0.061	0.553
F2 (max) (Hz)	**0.707**	-0.252	-0.001	0.130	-0.030	0.103	-0.363	0.056	0.014	0.136	0.075	0.150
F3 (max) (Hz)	**0.669**	-0.179	0.234	0.038	-0.088	-0.043	-0.204	-0.131	-0.104	-0.035	-0.026	0.154
F4 (max) (Hz)	**0.680**	0.112	0.289	0.012	0.000	0.002	-0.027	-0.257	0.068	0.012	-0.093	0.332
F5 (max) (Hz)	**0.656**	0.214	0.098	-0.156	-0.063	0.003	-0.006	-0.325	0.024	-0.090	-0.014	0.047
F6 (max) (Hz)	**0.726**	0.212	0.069	-0.122	0.194	0.033	-0.005	-0.224	0.068	-0.150	-0.002	-0.100
F7 (max) (Hz)	**0.585**	0.340	-0.095	-0.167	0.043	0.220	-0.009	-0.315	0.067	-0.102	-.0024	-0.217
F8 (max) (Hz)	**0.533**	0.270	-0.130	-0.198	-0.029	0.335	-0.205	-0.315	-0.108	-0.018	-0.043	-0.053
Df(Min) (Hz)	0.095	0.794	-0.285	-0.359	-0.010	0.075	.0172	0.185	-0.043	0.078	0.001	0.175
Max VTL (cm)	-0.095	-0.795	0.286	0.357	0.011	-0.076	-0.172	-0.187	0.044	-0.076	-0.001	-0.176
Q25% (Hz)	-0.323	-0.037	0.406	-0.294	0.225	0.386	0.124	-0.023	0.075	-0.013	0.171	-0.014
Q50% (Hz)	0.015	**-0.731**	0.269	-0.139	0.197	0.225	0.020	0.119	-0.041	0.136	0.148	-0.007
Q75% (Hz)	0.154	**-0.754**	0.219	-0.048	0.091	-0.027	0.006	0.129	0.011	0.224	0.061	-0.038
AM Var (dB/s)	0.241	-0.063	-0.143	0.619	0.207	0.196	0.381	-0.193	-0.198	0.077	-0.162	0.026
AM rate (s-1)	0.155	-0.019	-0.381	0.484	-0.316	-0.363	0.155	0.035	0.152	-0.114	-0.056	0.112
AM extent (dB)	-0.027	-0.093	0.194	0.057	0.374	**0.561**	0.036	-0.162	-0.295	0.304	-0.237	0.189
Duration (s)	0.438	-0.379	-0.353	0.042	-0.044	-0.010	0.315	0.002	0.310	-0.016	0.116	-0.042

There were significant differences among individual calves in PC scores (MANOVA: $F_{117, 783} = 3.07$, $p < 0.0001$). There was also a significant effect of age on the PC scores ($F_{9, 103} = 35.05$, $p < 0.0001$). In particular, formant frequencies (PC1) decreased strongly with age (Fig. 2.5). In contrast, however, there was no obvious effect of age on F0 (PC2, Fig. 2.6). Finally, there was a significant effect of the interaction between individual and age: calves vocalizations changed in different ways as they got older ($F_{117, 783} = 2.69$, $p < 0.0001$; see Fig. 2.5). There was no statistical effect of sex on the characteristics of calf calls (MANOVA: $F_{4,1} = 1.87$, $p = 0.286$).

As in calves, there were significant differences among cows in PCs scores for LFCs (MANOVA: $F_{63, 304} = 4.26$, $p < 0.0001$) and HFCs (MANOVA: $F_{168, 1032} = 2.93$, $p < 0.0001$).

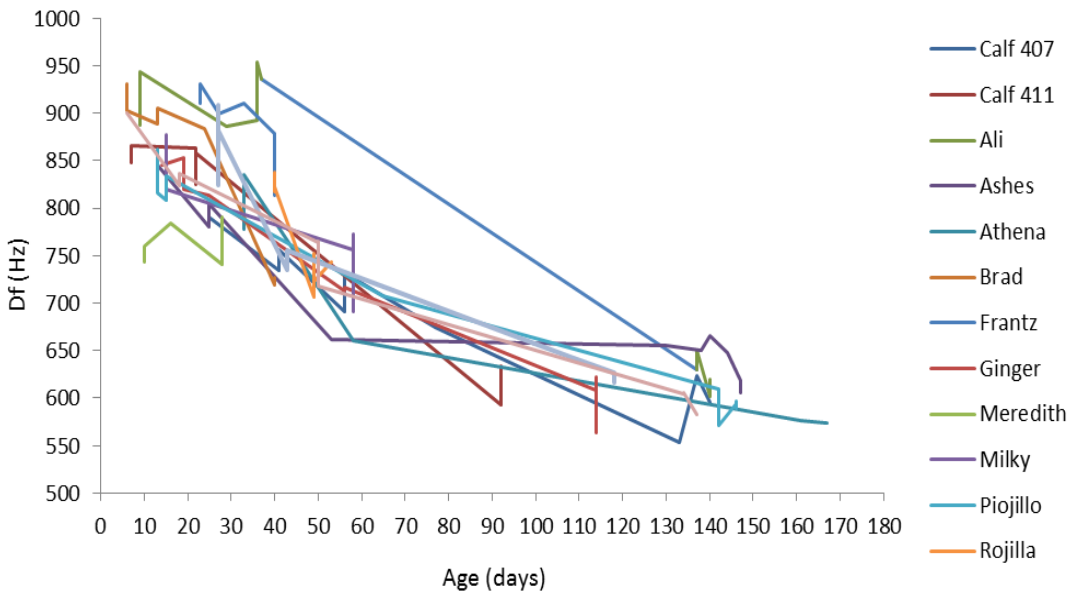

Figure 2.5. The effect of calf age on Df(min). Each line represents data from a different individual. MANOVA with PC1 (which was strongly correlated with Df(min)) as the response variable confirmed that the effect of age, and the interaction between age and individual, were significant ($F_{(1,111)} = 299.671$, $p < 0.001$).

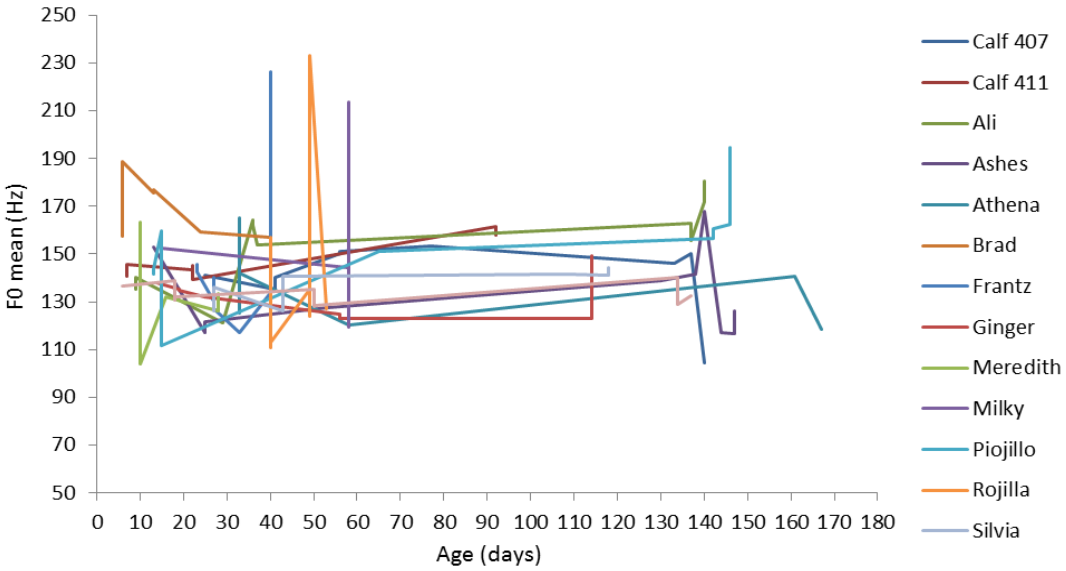

Figure 2.6. The effect of calf age on F0 mean. Each line represents data from a different individual. MANOVA with PC2 (which was strongly correlated with F0) as the response variable confirmed that there was no significant effect of age ($F_{(1,111)} = 3.050$, $p = 0.084$).

For calf calls, DFA produced three discriminant functions (DFs) which can be used to discriminate among individual calls (Table 2.9). For cow LFCs, DFA produced five significant discriminant functions (Table 2.10). For cow HFCs, DFA produced six significant discriminant functions (Table 2.11). For calf calls and cow HFCs, DF1 was highly correlated with PC1 (i.e. formant frequencies), PC2 (i.e. attributes of the F0 contour), and in HFCs also with PC3 (i.e. F0). In contrast, for cow LFCs, DF1 correlated mostly strongly with PC3 (which in turn correlated with the frequency value at the energy quartiles) and PC4 (which was correlated with other attributes of F0). Plots of calls and cross-validation

suggest that cow LFCs are relatively more individually distinctive, while calf calls are relatively less distinctive (Figures 2.7 – 2.9).

Table 2.9 Discriminant function coefficients for calf calls. Details of PCs are given in Table 2.6. Bold type indicate coefficients of magnitude 0.5 and above.

	DF1	DF2	DF3
Wilk's-λ	0.129	0.241	0.374
df	117	96	77
p	< 0.0001	< 0.0001	< 0.001
PC1	**0.682**	-0.310	0.138
PC2	**0.748**	0.267	0.016
PC3	-0.144	**0.687**	-0.150
PC4	-0.010	**-0.619**	0.135
PC5	-0.165	-0.212	**-0.518**
PC6	0.208	0.285	**0.646**
PC7	-0.480	-0.142	**0.604**
PC8	-0.143	0.241	-0.085
PC9	-0.212	0.156	0.300

Table 2.10 Discriminant function coefficients for cow LFCs. Details of PCs are given in Table 2.7. Bold type indicate.

	PC1	PC2	PC3	PC4	PC5
Wilk's-λ	0.028	0.094	0.234	0.470	0.655
df	63	48	35	24	15
p	< 0.0001	< 0.0001	< 0.0001	0.006	0.048
PC1	**-0.562**	-0.310	**0.610**	0.022	-0.202
PC2	**0.666**	-0.370	0.355	-0.513	0.228
PC3	**0.653**	**0.569**	0.230	0.076	-0.363
PC4	-0.196	0.448	0.259	-0.001	0.498
PC5	0.082	-0.083	0.216	0.082	0.469
PC6	-0.271	**0.596**	-0.199	-0.472	0.296
PC7	0.160	0.263	0.165	0.026	0.062
PC8	-0.409	0.350	0.**544**	-0.149	-0.118
PC9	0.083	0.154	0.393	0.321	-0.002
PC10	-0.182	-0.178	0.061	0.112	0.392
PC11	0.245	-0.097	0.193	0.409	0.207
PC12	0.209	0.123	-0.053	**0.541**	0.354

Table 2.11 Discriminant function coefficients and significances for cow HFCs. Details of PCs are given in Table 2.8. Bold type indicates coefficients of magnitude 0.5 and above.

	DF1	DF2	DF3	DF4	DF5	DF6
Wilk's-λ	0.028	0.069	0.137	0.233	0.351	0.506
df	168	143	120	99	80	63
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.001	0.048
PC1	-0.445	-0.027	**0.871**	-0.024	0.082	0.161
PC2	0.418	**0.869**	0.129	0.332	0.121	0.101
PC3	**0.718**	-0.142	0.166	**-0.585**	0.038	0.007
PC4	**0.954**	-0.105	-0.129	-0.111	0.087	0.231
PC5	0.341	0.050	0.177	0.275	**-0.700**	-0.034
PC6	0.475	**-0.606**	0.278	**0.500**	0.344	-0.300
PC7	-0.191	-0.143	-0.197	0.116	0.451	**0.665**
PC8	-0.128	**0.507**	-0.056	-0.265	0.437	**-0.518**
PC9	-0.257	-0.123	**-0.632**	0.287	0.016	-0.061

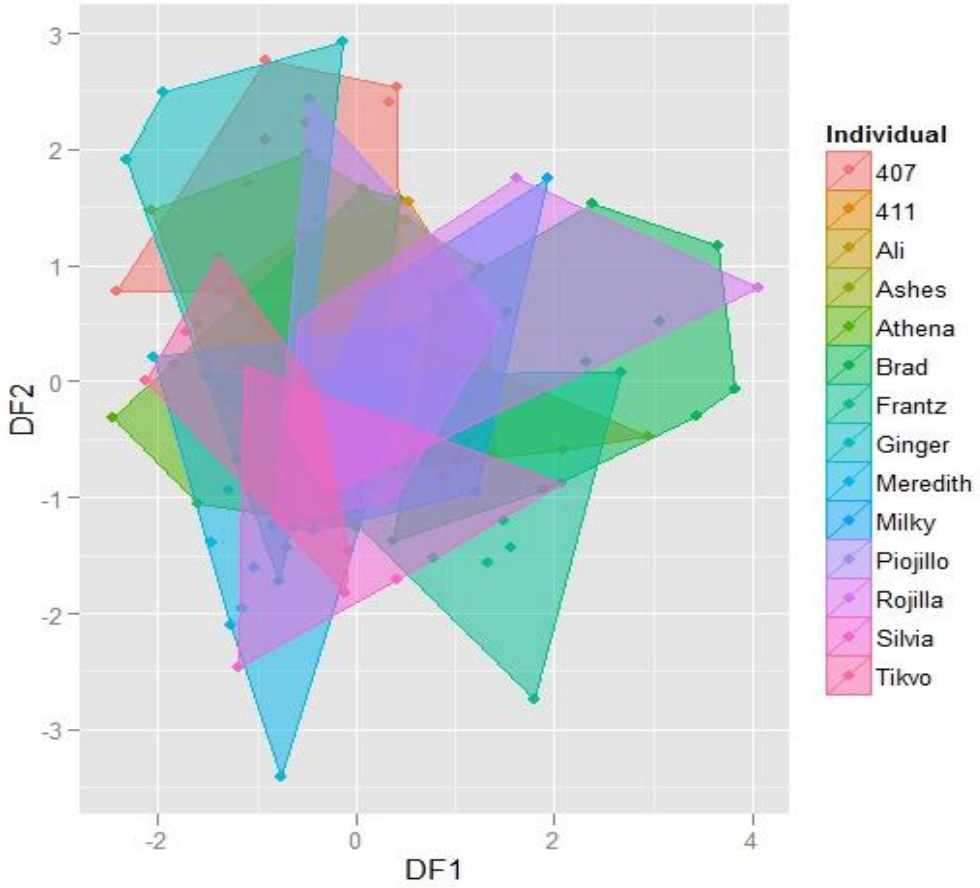

Figure 2.7. First two discriminant function scores for calf calls; n = 14 individuals, with 10 calls per individual. Points represent individual calls, while polygons delineate areas of parameter space occupied by calls of different individuals. Cross-validation classified 23.7 % of calls correctly.

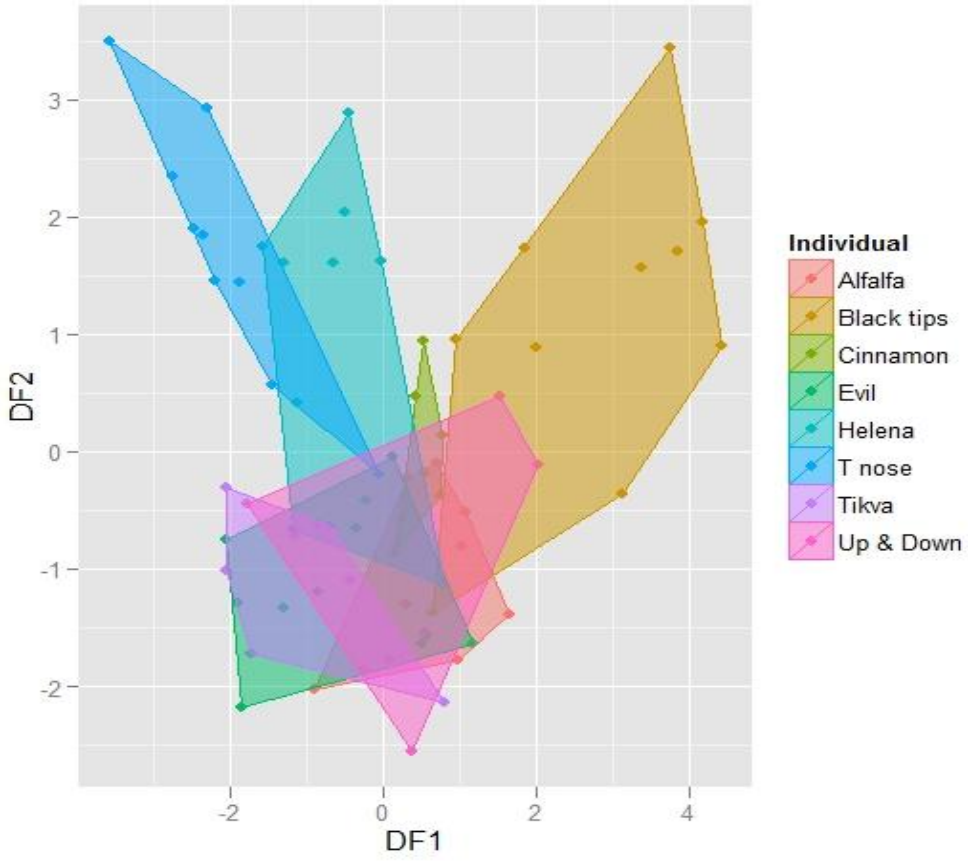

Figure 2.8. First two discriminant function scores for cow LFCs; n = 8 individuals, with 10 calls per individual. Points represent individual calls, while polygons delineate areas of parameter space occupied by calls of different individuals. Cross-validation classified 53.6 % of calls correctly.

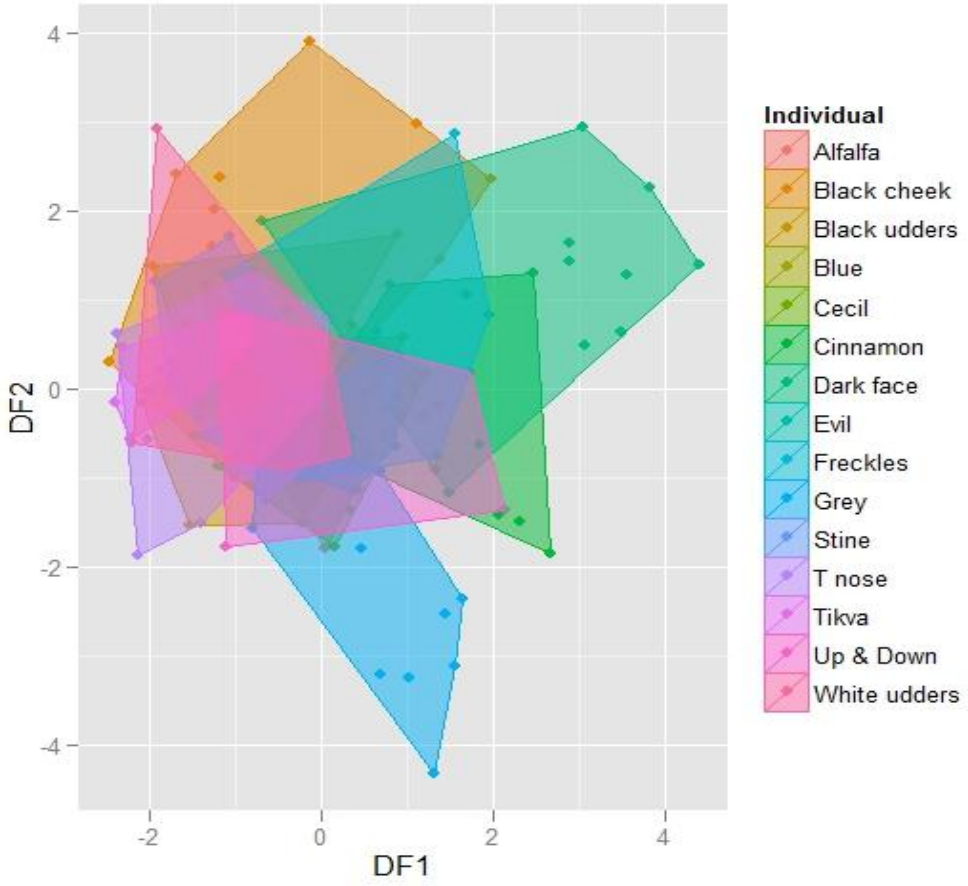

Figure 2.9. First two discriminant function scores for cow HFCs; n = 15 individuals, with 10 calls per individual. Points represent individual calls, while polygons delineate areas of parameter space occupied by calls of different individuals. Cross-validation classified 30.9 % of calls correctly.

Two additional DFAs with female and male data separately (to remove the potentially confounding effect of sex in calf calls) produced for both sexes two discriminant functions which can be used to discriminate among individual calls (Tables 2.12 and 2.13).

Table 2.12 Discriminant function coefficients for female calf calls. Bold type indicates components > 0.5.

	DF1	DF2
Wilk's-λ	0.136	0.318
df	45	32
P	< 0.0001	0.003
PC1	-0.380	-0.282
PC2	0.384	0.042
PC3	0.**791**	-0.150
PC4	-0.400	0.208
PC5	-0.148	-0.065
PC6	**0.507**	0.458
PC7	0.107	**0.820**
PC8	0.291	-0.454
PC9	0.112	0.069

No statistical differences were found when I compared the slopes of the relationship between age and the first two PCs between males and females (PC1: t = -0.114, df = 12, p = 0.198; PC2: t = -0.228, df = 12; p = 0.262).

Table 2.13 Discriminant function coefficients for male calf calls. Bold types indicates components > 0.5.

	DF1	DF2
Wilk's-λ	0.062	0.364
df	63	48
P	< 0.0001	0.016
PC1	**-0.581**	0.282
PC2	**-0.852**	-0.159
PC3	0.201	-0.349
PC4	0.172	0.450
PC5	0.264	**-0.505**
PC6	-0.065	**0.542**
PC7	**0.623**	0.334
PC8	0.092	0.110
PC9	0.308	0.298

2.5 Discussion

In this study I characterised in detail for the first time calls made between cows and their calves in a semi-natural setting. I was able to distinguish two different types of cow contact calls associated with different behavioural contexts, and with different acoustic structures. Low Frequency Calls (LFCs), were made with the mouth closed or only partially opened. They were extremely quiet, with a clear harmonic structure and an F0 average of 81.17 ± 0.98 Hz. LFCs were produced by mothers exclusively when they were in close proximity to their calves, in the two first weeks after birth. By contrast, High Frequency Calls (HFCs) were typically much louder, and were clearly audible in the field. HFCs present clear harmonic structure and an F0 average of 152.81 ± 3.10 Hz. In this type of call, the cows' mouths were fully opened for at least part of the call (the call sometimes started with the mouth only partially opened; see also Kiley 1972). HFCs were observed in both cows and calves when they were separated (e.g. in different fields) and they were looking for each other, usually for nursing. Although it has previously been suggested that cattle contact calls are individually distinctive (Kiley 1972; Barfield et al. 1994; Keyserlingk & Weary 2007), to my knowledge, my study is the first to describe in detail the differences from a source-filter theory perspective (Fant 1960; Titze 1994). The acoustic analysis of cow HFCs and LFCs, and of calf calls, indicates that both cows and calves produce individually distinctive vocalizations, as has been reported for other "weak hider" ungulates (see below; Briefer & McElligott 2011a). This finding has important implications for

our understanding of the directionality of individual recognition in cattle (see Chapter 3). There was, nevertheless, considerable overlap in the acoustic properties of the calls of different individuals, especially in the case of the calves. In addition, there was a significant age effect on calf vocalizations, with formant frequencies in particular decreasing with age, while parameters associated with F0 did not change.

2.5.1 Individuality in vocalisations

Individual recognition occurs when one organism identifies another according to its individually distinctive characteristics (Tibbetts & Dale 2007). In gregarious species that breed in large, high-density colonies, recognition between parents and offspring using vocal cues is especially likely to occur (e.g. Atlantic warlrus, *Odobenus rosmarus rosmarus*, Charrier et al. 2010; sheep, *Ovis aries*, Sebe et al. 2007; goat, *Capra hircus*, Briefer & McElligott 2011a). In this study, I have shown from a source-filter theory perspective (Fant 1960; Titze 1994) that contact calls produced from both cows and calves are individually distinctive. Vocal cues to individuality result from inter-individual differences in the vocal production anatomy/physiology or in the way it is operated by each individual (Vannoni & McElligott 2007; Taylor & Reby 2010). My results showed that filter-related vocal parameters (formant frequencies) and source-related parameters (those related to the fundamental frequency) were both important cues to determine individual

identity, although the ones with higher PIC scores were the source-related parameters (for detail see Table 2.5).

Vocal parameters with enough individuality can potentially be used as "signatures" for individual recognition (Shapiro 2009). The presence of many such parameters in cattle contact calls (nearly all the measured parameters had PIC > 1) is contrary to what would be expected considering that cattle has been classified as a “hider” species (Langbein & Raasch 2000). Hider species, in which offspring remain hidden in the vegetation whilst their mothers forage, are expected to show low individuality in offspring calls and strong individuality in mother calls, which leads to unidirectional mother-offspring recognition (e.g. fallow deer, *Dama dama*, Torriani et al. 2006). By contrast, follower species, in which offspring follow their mothers during foraging, show strong individuality in both mothers and offspring, and mutual vocal recognition, which seems to be essential in order to avoid misdirected maternal care (e.g. sheep, Sèbe et al. 2007). Cattle may not fall into either of these categories, and may be better described as “weak hider” species, with hiding behaviour only being evident in the first few days after birth (Le Neindre 1984). The fact that calves seem to display following behaviour relatively soon after birth, and the social integration with other conspecifics which this entails, might mean that selection has favoured individual vocalizations (for a full discussion of the distinction between hider and follower species, and its evolutionary implications, see discussion, Chapter 3).

Thus, although it is true in my study that individuality in calf calls was rather lower than in cows calls (LFCs in particular), in general my results suggest that cattle show a similar pattern to the one which is displayed in follower species and in other domestic ungulate weak hider species as goats (Briefer & McElligott 2011a).

Although previous studies have suggested the existence of individual differences in cattle vocalizations (e. g. five different types of calls in cows, bulls, and calves, Kiley 1972; in bulls, Hall et al. 1988; contact calls, Barfield et al. 1994), none have examined in detail the acoustic characteristics of calls in the context of the source-filter framework. Among the previous studies, the most complete attempt to characterize cattle vocalizations is the study carried out by Kiley (1972). She classified cattle vocalizations as belonging to five different types, each of which was composed of a combination of five distinctive syllables that were differentiated by their acoustic parameters (F0, amplitude and tonality). Among the repertoire of cattle calls, I exclusively considered contact calls in this study, which are the most likely to contain essential information about individuality needed for mother-offspring recognition (Briefer & McElligott 2011a). The classification of LFCs in my study (mean F0 = 81.17 ± 0.98 Hz) is consistent with what Kiley (1972) described as an "mm" call (mean F0 = 83 Hz) formed by two repetitions of the syllable "m", although I saw no evidence of any obvious syllable structure. As in my study, this type of call was determined by Kiley (1972) to be produced with a closed

mouth and was observed in the behavioural context of contact between mother and calf, and calls of this type have previously been suggested to be important in mother-offspring recognition (Kiley 1972; Barfield et al. 1994; Keysrlingk & Weary, 2007; Tucker 2009). Nevertheless, Kiley (1972) also reported this type of call from bulls and calves, which contrasts with my finding that neither calves nor bulls produced such low frequency calls in the field. Unfortunately the other types of calls that Kiley (1972) identified had a very broad acoustic description (e.g. F0 ranged from 50 to 800 Hz), were reported to be produced in almost any behavioural context (e.g. fear, isolation, pain, stress, etc.), and hence cannot easily be compared with the calls that I describe here.

Although, the results of this study provide evidence that cow and calf vocalizations are individually distinctive from a source-filter theory perspective, the cross-validated DFA of calf calls correctly classified just 23.7 % of calls (n = 14, with 10 calls per individual, chance level = 7.14 %), which is relatively low and even lower than the correct classification rate for hider species, such as fallow deer fawns (32.1 %, Torriani et al. 2006). One possible explanation is that calf vocalizations were changing during the course of the study. Given that the individuality analysis was based on a sample of calls recorded over a period during which calves are growing rapidly, age-related variation in call characteristics could partially mask the differences among individuals. A significant age effect was found in calf calls, where PC1 (which

correlated most strongly with formant frequencies) decreased as the calves got older (Figure 2.4). In contrast, there was no obvious effect of age on PC2 (which correlated most strongly with F0). These results are presumably a consequence of the fact that body size, and hence the size of the organs involved in sound production, increases with age. According to the source-filter theory, formant frequencies are determined by the length and shape of the cavities of the vocal tract or pharynx, mouth and nasal cavities (Fant 1960; Titze 1994). The vocal tract grows with the rest of the body as an animal matures and its length is directly dependent on body size (Taylor & Reby 2010). Because of this, it has been shown that there is a negative relationship between the frequency spacing between successive formants and body size among adult individuals of several species (red deer, *Cervus elaphus*, Reby & McComb 2003; fallow deer, Vannoni & McElligott 2008) and in juveniles as they grow (goats, Briefer & McElligott 2011b). The age-related changes in formant frequencies observed in calves in this study are thus probably the result of the development of the vocal tract during developmental growth. Unlike filter-related formant frequencies, source-related parameters, and in particular F0 values, are typically thought to correlate weakly with body size, although they can be good indicators of age and sex (Fitch 1997; Reby & McComb 2003). Differences in source-related characteristics of the call are determined by variation in sub-glottal pressure and in the length and shape of the vocal folds and their tension (Titze 1994). Because the relevant tissues are soft and unconstrained by skeletal structures, the sounds they produce do not

vary predictably with increasing body size (Fitch 1997). This may explain the absence of an age effect (a proxy for a body size effect) on the acoustic parameters associated with F0 in calves.

The relatively strong individuality observed in cow LFCs (cross-validation classified 53.6 % of calls correct, n = 8 with 10 calls individual; chance level = 12.5 %) is consistent with previous suggestions that the function of cow LFCs is as a mother-offspring recognition signal (Kiley 1972; Barfield et al. 1994; Keysrlingk & Weary, 2007; Tucker 2009). Similar classification efficiency has been recorded in contact calls of other mammals (e.g. 64 % for adult females in Atlantic Walrus, Charrier et al. 2010; 60 % for adult African elephants, *Loxodonta africana*, Soltis et al 2005; and 69.9% in adult female goats, Briefer & McElligott 2011a). In contrast, cross-validation of the cow HFCs classified only 30.9 % of calls correctly (n = 15 with 10 calls per individual, chance level = 6.67 %). This low value observed in HFCs could possibly be the due to the fact that LFCs, which are typically produced by cows very soon after calving, are the most important recognition signal for calves. It has been shown in cattle that there is very early individual recognition by offspring of their own mother's calls in cattle (Barfield et al. 1994), and in other ungulates such as goats (Briefer & McElligott 2011b). Once the calf has learnt to recognize its mother's identity through LFCs, it may be easier for calves to differentiate among cow HFCs without the need for such marked acoustic individuality.

It is important to note that the present study was performed with individuals in an open-field context, without intensive human contact or the presence of stressors such as artificial isolation. The few studies measuring cattle vocalizations before this one have been done in the context of intensive agricultural management (Grandin 1998; Watts & Stookey 1999; Weary & Chua 2000; Thomas et al. 2001; Ikeda & Ishii 2008) with a focus on identifying indicators of stress, rather than on the biology of the vocalizations themselves. In order to find possible acoustic indicators of welfare or productivity in cattle vocalizations, a thorough "baseline" description of vocalizations produced in natural behavioural contexts is of fundamental importance. Despite the fact that it has often been proposed that vocalizations might be good indicators of animal welfare (Grandin 1998; Watts & Stookey 1999; Watts & Stookey 2000; Maneuffel et al. 2004; Ikeda & Ishii 2008), nobody had performed a systematic study from the source-filter theory perspective before.

2.6 Conclusion

The results of this study provide evidence of individualized cow and calf vocalizations in the context of mother-offspring communication. I have identified the key sources of variation in unstressed cow and calf vocalizations, including source-related parameters (F0 and associated variables) and the filter-related parameters (mostly describing format frequencies). The latter features are variable in calves during development due to the lengthening of the vocal tract. This study can serve as a baseline for future studies of bovine communication, and the role of vocalisations as an indicator of wellbeing in animal welfare research.

2.7 References

Barfield, C. H., Tang-Martinez, Z., Trainer, J.M. 1994. Domestic calves (Bos taurus) recognize their own mothers by auditory cues. Ethology 97, 257-264.

Boersma P. & Weenink D. 2009. Praat: doing phonetics by computer. http:www.praat.org/

Bradbury, J. W., & Vehrencamp, S. L. 1998. Principles of Animal communication. Sinauer, Sunderland, MA.

Briefer, E., McElligott, A. G. 2011a. Mutual mother-offspring vocal recognition in an ungulate hider species (*Capra hircus*). Animal Cognition 14, 585-598.

Briefer, E., McElligott, A. G. 2011b. Indicators of age, body size and sex in goat kid calls revealed using the source-filter theory. Applied Animal Behavioural Science 133, 175-185.

Byrne, G., & Suomi, S. J. 1999. Social separation in infant *Cebus apella*: patterns of behavioural and cortisol response. International Journal Developmental Neuroscience 17, 265-274.

Charlton, B.D., Reby, D., & McComb, K. 2007. Female reed deer prefer the roars of larger males. Biology Letters 3, 382-385.

Charrier, I., Aubin, T., & Mathevon. 2010. Mother-calf vocal communication in Atlantic walrus: a first field experimental study. Animal Cognition 13, 471-482.

Fant, G. 1960. Acoustic theory of speech production. Mouton, The Hague.

Fischer, J. Hammerschmidt, K., Cheney, D.L., & Seyfarth, R. M. 2002. Acoustic features of males baboon loud calls: influences of context, age, and individuality. Journal of the Acoustical Society of America 1111, 1465-1474.

Fitch, W. T. 1997. Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. Journal of the Acoustical Society of America 102, 1213-1222.

Grandin, T. 1998. The feasibility of using vocalization scoring as an indicator of poor welfare during cattle slaughter. Applied Animal Behaviour Science 56, 121-128.

Hall, S.J.G., Vince, M., Shillito, E. & Garson, P.J. 1988. Vocalisations of the Chillingham cattle. Behaviour 104, 78-104.

Harrington, F.H. & Mech, L.D. 1979. Wolf howling and its role in territory maintenance. Behaviour 68, 207-249.

Ikeda, Y., & Ishii, Y. 2008. Recognition of two psychological conditions of a single cow by her voice. Computers and Electronics in Agriculture 62, 67-72.

Jones, G. 1999. Scaling of echolocation call parameters in bats. Journal of Experimental Biology 202, 3359-3367.

Kalko, E., K. V. 1995. Insect pursuit, prey capture and echolocation in pipistrelle bats (Microchiroptera). Animal Behaviour 50, 861-880.

Keyserlingk, M.A.G., & Weary, D.M. 2007. Maternal behaviour in cattle. Hormones and Behaviour 52, 106-113.

Kiley, M., 1972. The vocalisations of ungulates, their causation and function. *Z*eitschsrift für Tierpsychology 31, 171-222.

Langbein, J. & Raasch, M. L. 2000. Investigations on the hiding behaviour of calves at pasture. Archives fur Tierzucht-Archives of Animal Breeding 43, 203-210.

Le Neindre, P. and Sourd, C. 1984. Influence of rearing conditions on subsequent social behaviour of Friesian and Salers heifers from birth to six months age. Applied Animal Behaviour Science 12, 43-52.

Lingle, S., Rendall, D., & Pellis, S. 2007. Altruism and recognition in the antipredator defence of deer: 1. Species and individual variation in fawn distress calls. Animal Behaviour 73, 897-905.

Manteuffel, G., Puppe, B., & Schon, P. 2004. Vocalizations of farm animals as a measure of welfare. Applied Animal Behaviour Science 88, 163-182.

Marchant, J. N., Whittaker, X., & Broom, D. M. 2001. Vocalizations of the adult female domestic pig during a standard human approach test and

their relationships with behavioral and heart rate measures. Applied Animal Behavioral Science 72, 23-39.

Marchant-Forde, J. N., Marchant-Forde, R. M. & Weary, D.M. 2002, Responses of dairy cows and calves to each other´s vocalisations after early separation. Applied Animal Behaviour Science 78, 19-28.

Mellinger, D. K., & Clark, C. W. 2003. Blue whale (*Balaenoptera musculus*) sounds from the North Atlantic. Journal of the Acoustical Society of America 114, 1108-1119.

McComb, K. 1991. Female choice for high roaring rates in red deer, *Cervus elaphus*. Animal Behaviour 41, 79-88.

McComb, K., Packer, C., & Pusey, A. 1994. Roaring and numerical assessment in contest between groups of female lions, *Panthera leo*. Animal Behaviour, 47, 379-387.

McComb, K, & Reby, D. 2005. Vocal communication networks in large terrestrial mammals. In: McGregor, P. K (Ed.) Animal communication Networks. Cambridge Press. pp. 372-389.

McComb, K., Reby, D., Baker, L., and & Sayialel, S. 2003. Long distance communication of social identity in African elephants. Animal Behaviour. 65, 317-329.

McComb, K., & Reby, D. 2009. Communication in terrestrial mammals. In: Squire, L. R. (Ed.) Encyclpedia of Neuroscience, vol. 2. Oxford Academic Press. pp. 1167-1171.

McElligott, A. G., & Hayden, T. 1999. Context-related vocalization rates on fallow bucks, *Dama dama*. Animal Behaviour 58, 1095-1104.

McElligott, A. G., Birrer, M. & Vannoni, E. 2006. Retraction of the mobile descended larynx during groaning enables fallow bucks (*Dama dama*) to lower their formant frequencies. Journal of Zoology 270, 340-345.

McGarigal K., Cushman S. & Stafford S. 2000 Multivariate statistics for wildlife and ecology research. Springer, New York.

Newton-Fisher, N., Harris, S., White, P., & Jones, G. 1993. Structure and function of red fox *Vulpes vulpes* vocalizations. Bioacoustics 5, 1-31.

Owren, J., Seyfarth, R., & Cheney, D. 1997. The acoustic features of vowel-like grunt calls in chacma baboons (*Papio cyncephalus ursinus*): implications for production processes and functions. Journal Acoustical Society of America 101, 2951-2963.

Owings, D. H., Beecher, M., & Thompson, N. 1997. Communication. Perspectives in Ethology, Vol. 12. Plenum Press, New York.

Owings, D., & Morton, E. 1998. Animal vocal communication: a new approach. Cambridge University Press, Cambridge, UK.

Reby, D., Cangnelutti, B., & Hewison, A. J. M. 1999. Contexts and possible functions of barking in roe deer. Animal Behaviour 57, 1121-1128.

Reby, D., Joachim, J., Lauga, J., Lek, S., & Aulagnier, S. 1998. Individuality in the groans of fallow deer (*Dama dama*) bucks. Journal of Zoology 245, 79-84.

Reby, D., & McComb, K. 2003. Anatomical constrains generate honesty: acoustic cues to age and weight in the roars of red deer stags. Animal Behaviour 65, 519-530.

Reby, D., McComb, K., Cargnelutti, B., Darwin, C. J., Fitch, W. T., & Clutton-Brock, T. H. 2005. Red deer stags use formants as assessment cues during intra-sexual agonistic interactions. Proceedings of the Royal Society of London B 272, 941-947.

Rendall, D., Owren, M., & Rodman, P. 1998. The role of vocal tract filtering in identity cueing in rhesus monkeys (*Macaca mulatta*) vocalizations. Journal of Acoustic Society of America 103, 602-614.

Riede, T., & Fitch, T. 1999. Vocal track length and acoustics of vocalization in the domestic dog (*Canis familiaris*). Journal of Experimental Biology 202, 2859-2867.

Riede, T., Owren, M. J., & Arcadi, A.C. 2004. Nonlinear acoustics in pant hoots of common chimpanzees (*Pan troglodytes*): frequency jumps,

subharmonics, biphonation, and deterministic chaos. American Journal of Primatology 64, 277-291.

Robertson J.G.M. 1986. Male territoriality, fighting and assessment for fighting ability in the Australian frog *Uperoleria Rugosa.* Animal Behavioiur 34, 763-772.

Robisson P., Aubin T., & Bremond J. 1993. Individuality in the voice of the emperor penguin Aptenodytes fosteri: adaptation to a noisy environment. Ethology 94, 279-290

Searby, A., & Jouventin, P. 2003. Mother-lamb acoustic recognition in sheep: a frequency coding. Proceeding of the Royal Society B 270, 1765-1771.

Sebe, F., Nowak, R., Poindron, P., & Aubin T. 2007. Establishment of vocal communication and discrimination between ewes and their lamb in the first two days after parturition. Developmental Psychobiology 49, 375-386.

Semple, S., & McComb, K. 2000. Perception of female reproductive state from vocal cues in a mammal species. Proceedings of the Royal Society B 267, 707-712.

Seyfarth, R., Cheney, D., & Marler, P. 1980. Vervet monkey alarm calls: semantic communication in a free-ranging primate. Animal Behaviour 28, 1070-1094.

Shapiro, A. D. 2009. Recognition of individuals within the social group: signature vocalizations. In Brudzynky S. M. (Ed.) Handbook of mammalian vocalizations-an integrative neuroscience approach. Academic press, London, pp 495-503.

Simmons, A. M. 2003. Perspective and Progress in animal acoustical communication. In: Simmons, A. M., Popper, A. N., & Fay, R.R (Eds.) Acoustic communication. Springer, New York. pp. 1-14.

Sokal R. R., & Rohlf FJ. 1995. Biometry: the principles and practice of statistics in biological research. Freeman, New York

Soltis, J., Leong, K., Savage, A. 2005. African elephant vocal communication II: rumble variation reflects the individual identity and emotional state of callers. Animal Behaviour 70, 589-599.

Stehulova, I., Lidfors, L., & Spinka, M. 2008. Response of dairy cows and calves to early separation: Effect of calf age and visual and auditory contact after separation. Applied Animal Behaviour Science 110, 144-165.

Taylor A. M. & Reby D. 2010. The contribution of source-filter theory to mammal vocal communication research. Journal of Zoology 280, 221-236.

Taylor, A. M., Reby, D., & McComb, K. 2009. Context-related variation in the vocal growling behaviour of the domestic dog (Canis famliaris). Ethology 115, 905-915.

Theis, K.R., Greene, G. M., Benson-Amram, S. R., Holekamp, K. E. 2007. Sources of variation in the long-distance vocalizations of spotted hyenas. Behaviour 144, 5557-584.

Thomas, T. J., Weary, D. M., & Appleby M.C. 2001. Newborn and 5 - week - old calves vocalize in response to milk deprivation. Applied Animal Behaviour Science 74, 165-173.

Tibbetts, E. A., & Dale, J. 2007. Individual recognition: it is good to be different. Trends in Ecology and Evolution 22, 529-537.

Titze, I. 1994. Principles of voice production. Prentice Hall, Englewood Cliffs.

Torriani, M. V. G., Vannoni, E. & McElligott, A. G. 2006. Mother-young recognition in an ungulate hider species: a unidirectional process. American Naturalist 168, 412-420.

Tooze, Z. J., Harrington, F. H., & Fentresss, J. C. 1990. Individuality distinct vocalisations in timber wolves, *Canis lupus*. Animal Behaviour 40, 723-730.

Tucker, C.B. 2009. Behaviour of cattle. In: Jensen, P. (Ed). The Ethology of domestic animals. 2nd edition. CABI, UK. pp. 151-160.

Vanková, D., & Málek, J. 1997.Charracteristics of the vocalizations of red deer (*Cervus elaphus*) hinds and calves. Bioacoustics 7, 281-289.

Vannoni, E. 2007. Natural and sexual selection at work: the structure and function of fallow deer (*Dama dama*) vocalizations. PhD. thesis, University of Zurich.

Vannoni, E., & McElligott, A.G. 2007. Individual acoustic variation in fallow deer (Dama dama) common and harsh groans: a source filter theory perspective. Ethology 113, 223-234.

Vannoni, E., & McElligott, A.G. 2008 Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. PLoS ONE 3, e3013

Weary, D. M., & Chua, B. 2000. Effects of early separation of the dairy cow and calf. Separation at 6 h, 1 day and 4 days after birth. Applied Animal Behaviour Science 69, 177-188.

Weary, D. M., & Fraser, D. 1995. Calling by domestic piglets: reliable signals of need. Animal Behaviour 50, 1047-1055.

Watts, J. M., & Stookey, J. M. 1999. Effects of restraint and branding on rates and acoustic parameters of vocalization in beef cattle. Applied Animal Behavioural Science 62, 125-135.

Watts, J. M., & Stookey, J. M. 2000. Vocal behaviour in cattle: the animal's commentary on its biological processes of welfare. Applied Animal Behaviour Science 67, 15-33.

Wilden, I., Herzel, H., Peters, G., & Tembrock, G. 1998. Subharmonics, biphonation, and deterministic chaos in mammal vocalization. Bioacoustics 9, 171-196.

CHAPTER THREE

Mother-offspring vocal recognition in cattle

3. Mother-offspring vocal recognition in cattle

3.1 Introduction

The mother-offspring bond is crucial in mammals. Individual recognition in gregarious species is fundamental in order avoid misdirected parental investment (Trivers 1972; Nowak et al. 2000). In ungulates, two very different parental care strategies have been identified: the offspring of "follower" species remain with their mother while she forages, but "hider" species mothers leave their offspring concealed in vegetation (Lent 1974; Fisher 2002). Vocal communication is thought to play a central role in mother-offspring recognition in hider ungulates, but vocal communication in domestic cattle (*Bos taurus*), which is classified as a hider species (Hall et al. 1988; Langbein & Raasch 2000), has not been studied in detail. In order to determine if mother-offspring individual vocal recognition occurs in cattle and if that process is unidirectional or bidirectional, I conducted a set of playback experiments with cows and their calves in an open-field environment.

3.1.1 Parental care: mother-offspring relationship

The mother-offspring dyad or group is the basic social unit in mammals. Even in species that are solitary as adults, the bond between mother and offspring is usually very close (Vaughan et al. 2000).

In social mammals, individual recognition plays an important role in their social life and allows them to identify the species, sex, offspring, and social status of other individuals. Individual recognition can be defined as: a subset of recognition that occurs when one organism identifies another one according to its unique distinctive characteristics (Tibbetts & Dale 2007). This is achieved through several sensory modalities and is crucial for the survival of dependent offspring. Mothers that live and breed in large, high-density colonies, where the risk of misdirected parental care is high, need selective strategies in order to restrict lactation exclusively to their own offspring and hence maximise their developmental rate and chances of survival (Trivers 1972; Nowak et al. 2000). Very sophisticated recognition strategies are seen in many social mammals where, for example, mother and offspring are able to spend long periods of time out of sight and yet a refined parent-offspring vocal recognition process allows the dyad to find each other (e.g. Mexican free tail bats: *Tadarida brasiliensis mexicana*, Balcombe 1990; Australian fur seal: *Arctocephalus tropicalis,* Charrier et al. 2002; sheep: Ovis aries, Searby and Jouventin 2003; fallow deer: *Dama dama*, Torriani et al. 2006; walrus: *Odobenus rosmasus rosmasus,* Charrier et al. 2010; Australian sea lion: *Neophoca cinerea,* Pitcher et al. 2010; goats: *Capra hircus,* Briefer & McElligott 2011).

3.1.2 Vocal mother-offspring communication in ungulates

All ungulate offspring are precocial. They are characterized as giving birth to offspring that are well-developed morphologically, and potentially able to follow their mother shortly after birth. Newborns show a rapid development of inter-individual recognition, and mothers usually care exclusively for their own young (Nowak et al. 2000).

In ungulates, the recognition process between mother and their offspring involves vision (Coulon et al. 2007 & 2009), olfaction (Alexander 1977) and audition. However, vision is useful just in open habitats and olfaction cues only permit identification at short range (Lickliter & Heron 1984); while vocal signals are efficient over both, short and long distances and open and inconspicuous habitats. Therefore, vocal communication appears to be a key factor for mother-offspring recognition in gregarious ungulates (Searby & Jouventin 2003).

Vocalizations usually contain specific information such as species identity, individual identity, social context, and phenotypic traits of the vocalizing animal (Fischer et al. 2002; Reby & McComb 2003; Blumstein & Munos 2005). Individual recognition based on vocal signatures is common in mammals (Rendall et al. 1996; Frommolt et al. 2003), and heterogeneity among individuals is directly related to variation in the individual morphology of the

vocal apparatus, which influences the spectral structure of the emitted vocalization (Riede et al. 2005; McElligott et al. 2006).

Within the wide range of calls already identified in ungulates, those which are involved in parental-offspring communication care are usually called "contact calls" (Vannoni et al. 2005). These very common vocalizations between mothers and offspring are important for their behavioural interactions. Communication between mother and offspring represents a highly individualized process due to the strong bond between the pair involved. Mothers and their offspring emit contact calls mainly to find each other when they are separated (Vannoni et al. 2005).

3.1.3 Strategies for predator avoidance in ungulates: hider species vs. follower species

Two main strategies for avoiding predators in the first weeks of life have been observed in newborn ungulates: "hiding" and "following" (Lent 1974; Fisher et al. 2002). Hider offspring do not follow their mothers and spend most of their time hidden in vegetation in order to avoid potential predators. Mothers usually spend most of their time foraging at least 100 m away from their offspring's hiding place and they return intermittently to feed the offspring. Since hider offspring have sedentary habits, they maximize their growth rate and minimize their age at weaning. Because mothers bring milk the energetic

cost for them is minimum and they grow fast (Fisher et al. 2002). On the other hand, follower offspring are able to walk very soon after birth and therefore they are able to rely on maternal and group defence to avoid predators. Following offspring are able to suckle regularly because they spend most of the time near their mothers. The evolution of the follower strategy is thought to be favoured in open habitats, where there is no cover available which could provide protection from predators (Lent 1974). In such circumstances, group defence appears to be the best strategy in order to avoid predators. Even though it may be costly, group defence in the open can be very effective, and it has been shown that offspring survival is high in follower species (Ralls et al. 1986; Fisher et al. 2002). Therefore, in habitats where cover from predators is available, a different strategy is typically thought to be favoured (hiders) because the energetic and opportunity costs of group defence can be avoided (Fisher et al. 2002). It is possible that these two widely differing strategies may have affected the vocal recognition process of mothers and offspring because of the large differences in the way that mothers and offspring of hider species interact during the first weeks of life.

Because in hider species females memorise the approximate locations of their hidden offspring (Lent 1974; Torriani et al. 2006), there is little selection pressure on offspring to develop individualized calls or on the mother to identify her offspring's calls. However, the offspring should be able to identify their own mother to avoid being detected by predators and in order to initiate

nursing bouts. Therefore, hider species are expected to display low vocal individuality in offspring and strong individuality in mother calls, and a unidirectional recognition process of mothers by offspring, at least in early stages of offspring's life. In contrast, follower species live surrounded of many conspecifics (Fisher et al. 2002). Consequently development of strong vocal individuality in both mothers and offspring, in order to avoid misdirected maternal care, is predicted. There is some empirical support for these predictions. For example, it has been shown that female fallow deer (*Dama dama*), whose young hide themselves they are not put there by their mothers, have individualized contact vocalizations, but their offspring do not (Torriani et al. 2006). In the same study, playback experiments demonstrated that fawns can distinguish the calls of their mothers from those of other females, but mothers cannot recognise their own fawn's calls (Torriani et al. 2006). Consequently, it was determined that the vocal identification process is unidirectional in this particular species. By contrast, it has been shown that in follower species such as domestic sheep (*Ovis aries*), and reindeer (*Rangifer tarandus*) mothers and offspring are capable of recognizing each other using contact calls (Searby & Jouventin 2003; Sebe et al. 2007& Espmark 1971 & 1974, respectively).

Other studies have, however, contradicted the idea that the evolution of hider and follower strategies has led to a clear dichotomy in patterns of individual recognition in ungulates. For example, it is known that wild and feral goats (*Capra sp*.) show typical hider behaviour, where the offspring stay hidden for

up to six weeks (McDougall 1975; Allan et al. 1991), and hence they would be expected to display a unidirectional vocal recognition pattern between mother and offspring. A recent study on domestic goats has shown, however, that mothers and offspring have individually distinctive vocalizations, and that they are able to recognize each other even in the very first days after birth during the hiding phase (Briefer & McElligott 2011).

3.1.4 Maternal behaviour in cattle

In cattle, isolation to give birth is an important preliminary step in the formation of the mother-offspring bond as it protects the dyad from disturbances by other female cows and predators, and facilitates early interaction without interference. The modern artificial environment in farms is likely to suppress or alter much maternal behaviour in domestic cattle. Despite this, restlessness and a preference for isolation with a semblance of territoriality for a small area is still evident in domestic cattle (Arave & Albright 1981).

Domestic cattle are usually considered to display a hider strategy, despite the general absence of hiding behaviour in typical domestic settings (Watts & Stookey 2000; Jensen 2001; Tucker 2009). Langbein & Raasch (2000) found that calves spend three times longer lying down within high vegetation (that was artificially provided) than when in an open field, suggesting that the absence of hiding behaviour in domesticated cattle may largely be a result of

the lack of cover. Additionally, placentophagia (consumption of the placenta by the mother after giving birth) is a widely reported phenomenon in cattle (Jensen 2001). This could be considered to support the hypothesis that cattle naturally follow a hider strategy. As well as serving a nutritional function, placentophagia may have reduced the likelihood of detection of hidden calves by predators, and hence been an adaptation to the hider way of life in wild cattle (Edwards & Broom 1982; Tucker 2009).

3.1.5 Vocal communication and individual recognition in cattle

Playback studies in cattle have shown that calves are able to identify their own mother's vocalizations (Barfield et al. 1994; Marchant-Forde et al. 2002). Although these studies suggest individual recognition of mothers by calves, they did not assess recognition of calf vocalisations by their mothers, and it is not yet known therefore, whether parent-offspring recognition in this species is uni- or bidirectional. It has also reported that cows and calves become more vocal after being separated from each other, presumably due to stress (Stehulová et al. 2008). Playback studies without artificial isolation of mother-offspring and under more natural conditions have not been carried out yet.

3.2 Objectives

I carried out series of playbacks experiments in order to establish whether bidirectional individual recognition between mother and offspring in cattle occurs. Individual natural calls were recorded and used to construct artificial call sequences in such a way as to mimic those observed in the field. Subsequently, these sequences were played back to animals that were either directly Own (mother or offspring) to the individual making the call in the recording or not. The behavioural responses to these playbacks were scored and analysed to evaluate any differences between direct relatives and non-relatives, which might be indicative of specific mother-offspring recognition.

3.3 Methods

3.3.1 Study site and subjects

The study was carried out with two cattle herds situated in two separated fields (52 ha and 23 ha, respectively) on a farm in Nottinghamshire, UK (52° 93´ 72", 1° 06´ 09´´W), from February of 2010 to August 2010. For the playback experiments 44 individuals (cows; n=22; calves; n=22) were tested. Playbacks of calf calls to cows were all carried out between 5 to 10 days after the calf recordings were made. All individuals included in both studies were free to roam in the fields with food and water *ad libitum*. Calves included in this study were all born between February and August 2010, and all were sired by the same bull. The two herds were kept separately in their fields without interchange of animals, except in two situations. First, the owners considered that some individuals should be swapped between fields, in order to match cows with one of two bulls according to size. Second, isolation for medical treatments for periods up to two weeks was occasionally required for some animals. All the calves included in the study were kept all year long in the same field with their mothers.

3.3.2 Sound recording and signal acquisition

Recordings of individual cow and calf contact calls were made opportunistically between 8 am and 5 pm from February to August, 2010. Calls were recorded at distances of 10 - 30 m from the vocalizing animal with

a Sennheiser MKH70 directional microphone, connected to a MaranzT PMD660 digital recorder (sampling rate 44.1 kHz). Accurate, individual identification was done from specific ID tags placed in the animals' ears by the farmer and by visual recognition of coat markings. Because of the farm records, the exact ages of the calves at the moment that calls were recorded were known. For analysis of responses of cows to playbacks of calf calls, calf age at the moment of the playback was considered as an independent variable. Even though the recordings used were made on different days, they were never made more than 10 days before the playback trials were carried out. Unfortunately, a shortage of appropriate records meant that the age of the cows was not known, but all were at least 2 years old and hence fully grown. All calls were subsequently inspected, and only the high quality recordings (where the call was clear, with little background noise) were included for the playback experiments.

Vocalizations were uploaded to a computer at a sampling rate of 44.1 kHz and saved in a WAV format at 16-bit amplitude resolution. I used Praat v.5.0.47 DSP Package (Boersma and Weenink, 2009) to build the sequences up for playback experiments. Calls were individually visualized in spectrograms in Praat (FFT method, window length = 0.1 s, time steps = 100, frequency steps = 250, Gaussian window shape, dynamic range = 40 dB). Vocalizations with high levels of background noise (as visualized in the spectrogram) were not considered for playback experiments. Cows and calves can produce contact

calls with closed or open mouth and some calls with these two elements in the same call. In such cases, the original total call length was recorded and just the part of the call made with the mouth open was analysed.

3.3.3 Playback sequences

For the playback experiments, call sequences were designed to reflect the natural call sequences and calling rate observed in the field. With this aim in mind, all the available natural sequences from cows and calves were firstly visualized in spectrograms. The silence interval between each call, and number of calls made in a sequence were measured. Subsequently, the means of these parameters were used to build the artificial playback sequences, with a random selection of the individual calls with the best sound quality from each individual used as the building blocks. For cows, sequences of five calls interspersed with 2.7 s intervals of silence were used (Fig. 3.1). For calves, sequences of three calls interspersed with 2.8 s intervals of silence were used (Fig. 3.2). For cow playback sequences, high frequency calls (HFC) were used if possible. In a few cases (n = 2) where cows did not make HFC or it was not possible to get any high quality call recordings, it was necessary to use low frequency calls (LFC). In these rare cases the playbacks were made closer to calves in order to assure that they would hear the cow call sequence.

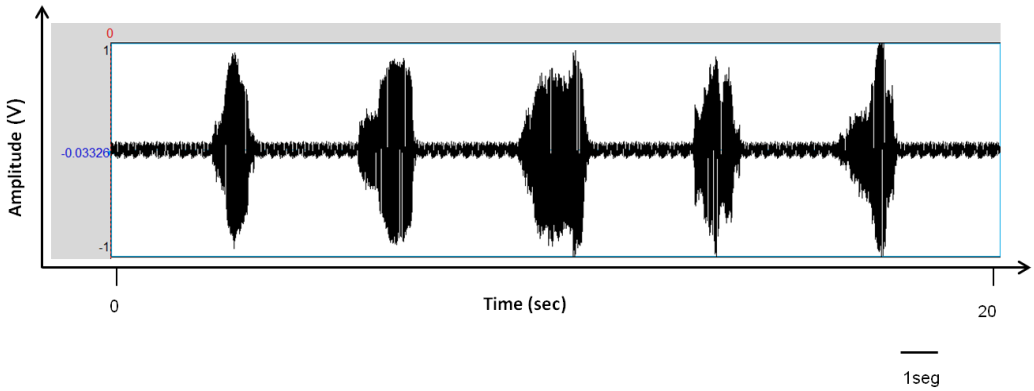

Figure 3.1. Waveform of a cow sequence artificially built up for playback experiments.

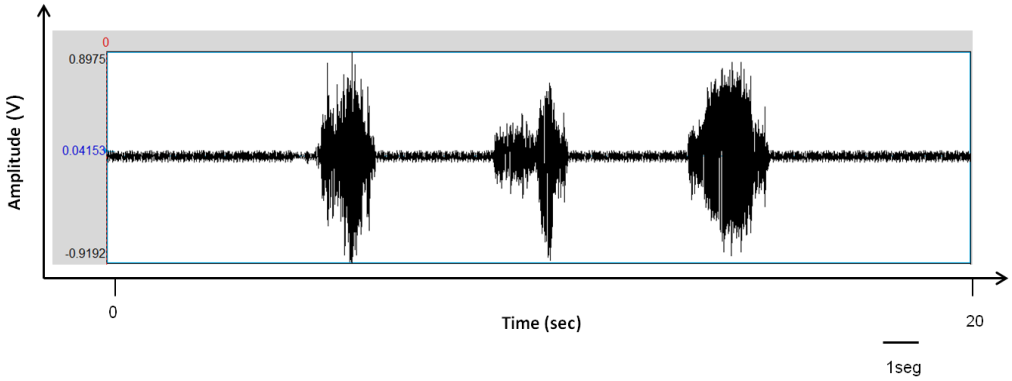

Figure 3.2. Waveform of a calf sequence artificially built up for playback experiments.

3.3.4 Playback procedure

All playback trials were performed opportunistically in the field, without any artificial isolation or manipulation of the animals, and while trying to cause the least disturbance possible. In each playback trial, the behavioural

responses of two individuals were filmed simultaneously: the “Own” individual was the individual directly related (mother/offspring) to the individual whose calls were being played from the loudspeaker in that particular trial. The "Other" individual was the nearest individual in the field that was not the offspring or mother of the calf/cow which provided the playback sequence. To avoid pseudo-replication every individual was tested just once (Kroodsma et al. 2001). It is important to note that all the animals tested were likely to be related to some extent. In the case of the calves, Other individuals shared a father with Own individuals. In the case of the cows, the pedigrees were unknown but it is likely that some individuals were half-sisters.

I played back call sequences, stored as mp3 files on a CD at sampling rate of 44.1 KHz, using a Skytronic TEC076 portable speaker system (frequency response: 50-20 kHz ± 3dB). Own and Other cows and calves were tested when their own mothers or offspring were not in direct line of sight and at least 30 m away from them, the aim being to avoid auditory and visual contact as much as possible. The loudspeaker was hidden with a camouflage tent, 10 – 30 m away from the animals being tested. Each trial was video recorded with two digital video cameras (a Sony DCR-SR58 and a Panasonic SDH-H80) set up 5 – 20 m from the tested animals.

3.3.5 Behavioural responses

The behavioural responses of cows and calves were assessed from videos of the playbacks and each Own and Other individual was allocated a response score indicating the strength of the observed reaction to the playback: (0) no reaction; (1) ear movements and/or looking towards loudspeaker; (2) directing body towards loudspeaker and/or standing up; (3) walking towards loudspeaker; (4) calling back and/or meeting real mother/calf. Stronger behavioural responses typically included elements typical of weaker responses (e.g. an individual which moved towards the loudspeaker [score 3] typically first pricked its ears, and looked at the loudspeaker [score 1] before standing [score 2]). Latency to respond was recorded as the time between the beginning of the playback and the first behavioural response with a score of 1 or above.

3.3.6 Statistical analysis

Differences in the strength of behavioural responses between treatments (Own vs. Other) were examined using generalized linear mixed models (binomial GLMMs) for cows and calves. Various measures of calf age were included as covariates in the models. When calves were receiving the playback, their own age was considered (number of days from birth until the moment of the trial). When cows were receiving playbacks, the age of their own calf (number of days from birth until the moment of the playback trial),

and the age of the calf which provided the call for the playback were considered. All GLMMs were analyzed using R v 2.13.0 (R Development Core Team 2009). The model fitting process involved the deletion of interactions and main effects from the full model, with the significance of the contribution of each term to the deviance explained being tested with a chi-squared test at the point of deletion. Age was fitted as a covariate, with different full models being constructed for the two possible measures of age in the case of cow responses. Differences between Other and Own cows and calves in the latency to react to the playbacks were assessed from the video recordings, and were analysed using a Wilcoxon signed-rank test. This analysis was carried out using SPSS v 20.

3.4 Results

I found strong evidence that cows were able to identify the calls of their own calves. For three of the four types of behavioural response recorded, mothers were significantly more likely to respond to calls from Own calves (their own calves) than to calls from Other calves (Fig. 3.3; Table 3.1).

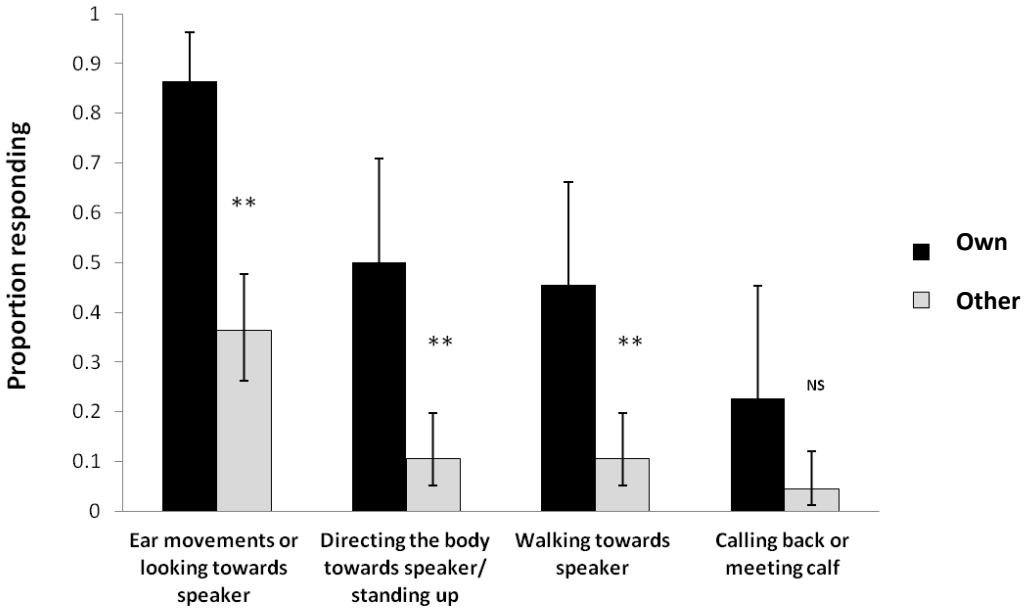

Figure 3.3. Proportion of cows responding to playbacks of their Own or a different (Other) calf. Four different behavioural responses were recorded, and these are presented in order of the strength of the reaction, with the strongest response being on the right (Binomial GLMM, ** $P < 0.01$, NS = non significant).

Table 3.1. Results of binomial generalised linear mixed models testing the effect of the identity of the cows listening to the call (Own or Other), the age of the calf providing the call (number of days from birth until the moment of the playback trial), and the interaction between the two, on the probability that cows would respond to playbacks of vocalisations in the field. Responses of four different types were considered. Because Other and Own animals were exposed to playbacks simultaneously, playback trial was fitted as a random effect.

Effect	Ear movements or looking towards speaker	Directing the body towards speaker /standing up	Walking towards speaker	Calling back or meeting calf
Animal (Own vs. Other)	$X^2 = 6.5988$ Df = 1 P = 0.0102	$X^2 = 7.518$ Df = 1 P = 0.0061	$X^2 = 5.9768$ Df = 1 P = 0.0145	$X^2 = 2.5482$ Df = 1 P = 2.5482
Age of playback calf	$X^2 = 0.6255$ Df = 1 P = 0.2382	$X^2 = 0.123$ Df = 1 P = 2.3793	$X^2 = 2.7949$ Df = 1 P = 0.0945	$X^2 = 1.9548$ Df = 1 P = 0.1621
Animal x Age of playback calf	$X^2 = 7.4327$ Df = 1 P = 0.0064	$X^2 = 0.4295$ Df = 1 P = 0.624	$X^2 = 0.3472$ Df = 1 P = 0.5557	$X^2 = 3e\text{-}04$ Df = 1 P = 0.9852

When the age of the calf providing the playback was considered (number of days from birth until the moment of the playback trial), there was no overall effect of the age on any of the behavioural responses (Table 3.1). However, there was a significant interaction between the identity of the animal (Own or Other) and the age of the calf used in the playback for one of the observed behavioural responses; cows were more likely to move their ears or look towards the speaker when hearing calls of younger calves, but only when the calf was their own (Fig. 3.4, Table 3.1). No such interaction was evident when the remaining behavioural responses were analysed (Table 3.1).

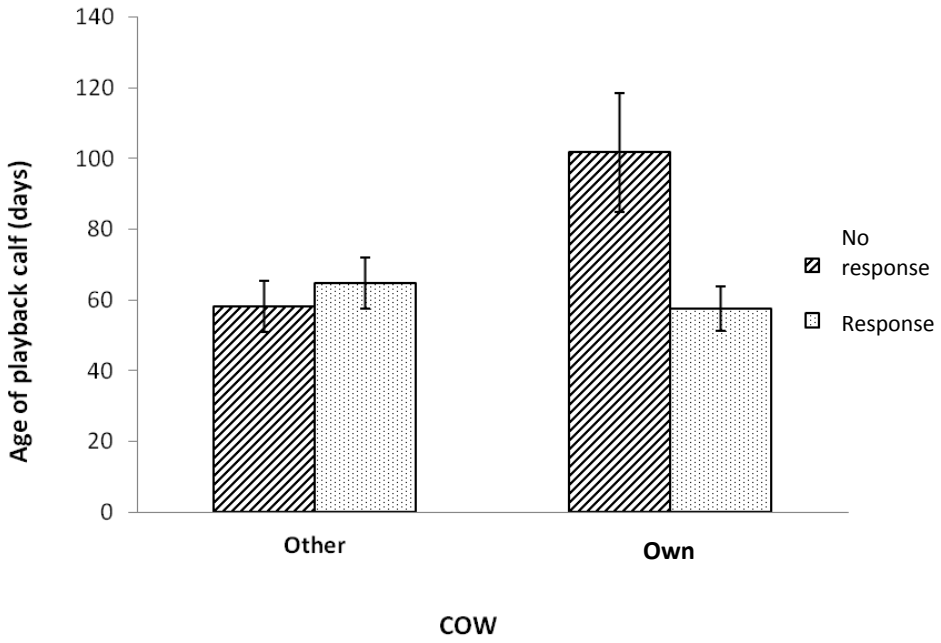

Figure 3.4. The effect of the age of the calf used in the playbacks trials on the likelihood that cows would respond to playbacks from their Own and Other calves. Data shown are the mean age (+/- SEM) of the calves whose calls were being played in cases where the cow hearing the calls either did or did not respond by moving her ears or looking towards speaker .

An alternative analysis of the effect of age, using the age of the cow's own calf as a covariate, produced similar results. There was a significant main effect of age on three of the four behavioural responses, with cows overall being more likely to respond to playbacks if their own calves were young (Table 3.2). However, this effect was primarily caused by the heightened response of cows to calls of their Own calves. If those calves were young there was a significant

interaction between age and the identity of the calling animal for all but one of the behavioural responses (Table 3.2; Fig. 3.5).

Table 3.2. Results of an alternative analysis to that shown in Table 3.1, considering the age of the calf belonging to the cow listening to the playbacks as a covariate, instead of the age of the calf providing the playback. In the case of Own cows, this age is the same as the age of the calf providing the call, but it is different for Other cows, whose own calves were not involved in the trial. Responses of four different types were considered (as explained in Section 3.3.5). Because Other and Own animals were exposed to playback simultaneously, playback trial was fitted as a random effect.

Effect	Ear movements or looking towards speaker	Directing the body towards speaker /standing up	Walking towards speaker	Calling back or meeting calf
Animal (Own vs. Other)	X2 = 6.5988 Df = 1 P = 0.0102	X2 = 7.518 Df = 1 P = 0.0061	X2 = 5.9768 Df = 1 P = 0.0145	X2 = 2.5482 Df = 1 P = 0.1104
Age of playback calf	X2 = 1.2688 Df = 1 P = 0.26	X2 = 11.431 Df = 1 P = 0.0072	X2 = 12.614 Df = 1 P = 0.0003	X2 = 5.8684 Df = 1 P = 0.0154
Animal x Age of playback calf	X2 = 6.0932 Df = 1 P = 0.0135	X2 = 11.036 Df = 1 P = 0.0089	X2 = 9.9311 Df = 1 P = 0.0016	X2 = 1.8181 Df = 1 P = 0.1775

Calf responses to calls from Own and Other cows were not as strikingly different as the responses of mothers to Own and Other calves, but there was nevertheless evidence that calves can identify their mothers' vocalisations. Calves were significantly more likely to move their ears or look towards the speaker in response to calls from Own females (their own mothers) than to calls from Other females (Fig. 3.6; Table 3.3). No significant differences were found for the other three behaviours (Table 3.3).

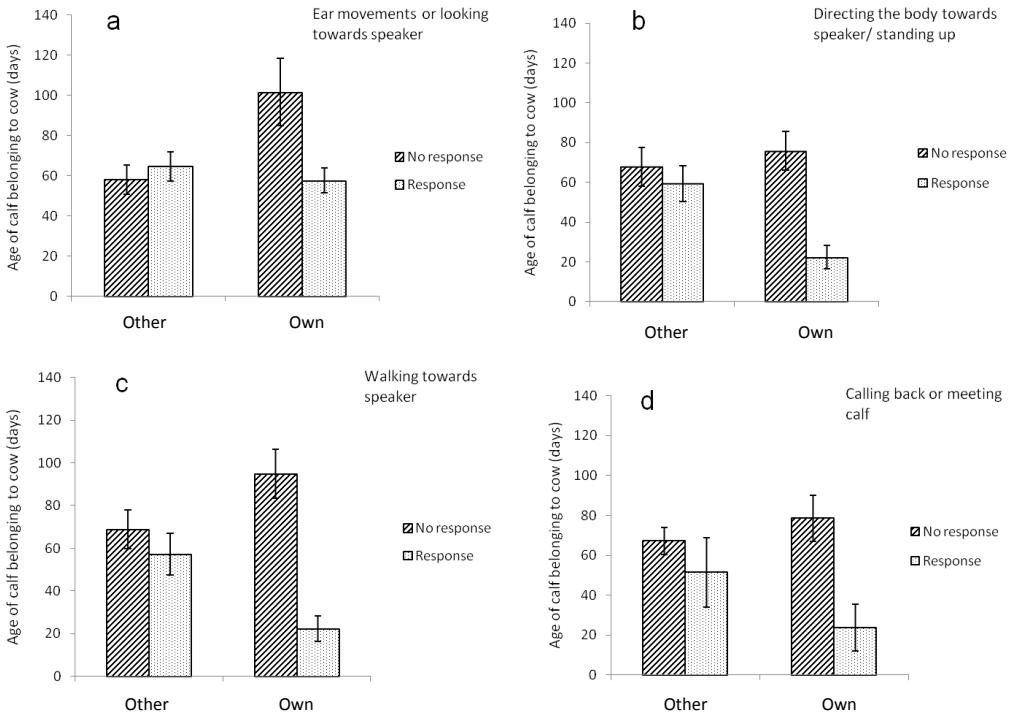

Figure 3.5. The effect of the age of a cow's calf on the likelihood that she would respond to playbacks from her Own and Other calves. Data shown are the mean age (+/- SEM) of the calves belonging to cows which either did or did not respond to playbacks in each of four different ways. The behavioural responses are presented in order of strength: **a** Ear movements or looking towards speaker. **b** Directing the body towards speaker or standing up. **c** Walking towards speaker. **d** Calling back or meeting their own calf.

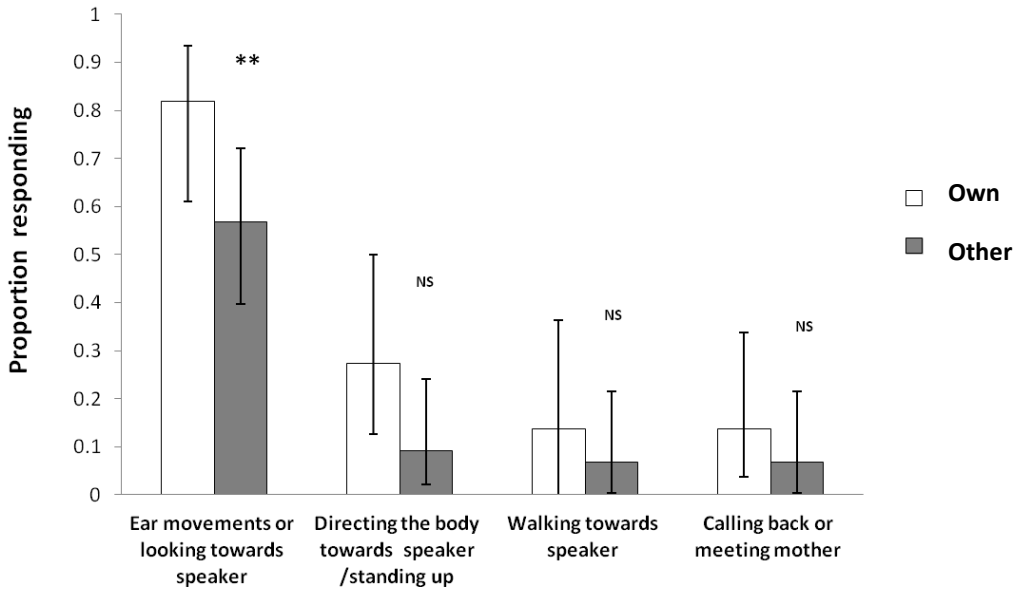

Figure 3.6. Proportion of calves responding to playbacks from Own and Other cows. Four different behavioural responses are presented in order of the strength of the observed reaction to the playback trial, from left to right (Binomial GLMM, **P < 0.01, NS = non significant).

There was no significant effect of calf age on the probability that it would show any of the observed behaviours in response to the playbacks, and neither was there an interaction between the identity of the animal (Own or Other) and age (Table 3.3).

Table 3.3. Results of binomial generalised linear mixed models testing the effect of the identity of the calves listening to the call (Own or Other), the age of the calf, and the interaction between the two, on the probability that calves would respond to playbacks of vocalisations in the field. Responses of four different types were considered (as explained in Section 3.3.5). Because Other and Own animals were exposed to playback simultaneously, playback trial was fitted as a random effect.

Effect	Ear movements or looking towards speaker	Directing the body towards speaker /standing up	Walking towards speaker	Calling back or meeting mother
Animal (Own vs. Other)	$X^2 = 5.9909$ Df = 1 P = 0.0143	$X^2 = 3.5534$ Df = 1 P = 0.0594	$X^2 = 0.7823$ Df = 1 P = 0.3764	$X^2 = 0.7823$ Df = 1 P = 0.3764
Age of playback calf	$X^2 = 0.6333$ Df = 1 P = 0.6333	$X^2 = 0.4693$ Df = 1 P = 0.4933	$X^2 = 1.1174$ Df = 1 P = 0.2905	$X^2 = 0.2905$ Df = 1 P = 0.2905
Animal x Age of playback calf	$X^2 = 0.0982$ Df = 1 P = 0.754	$X^2 = 0.2571$ Df = 1 P = 0.6121	$X^2 = 0$ Df = 1 P = 0.997	$X^2 = 0$ Df = 1 P = 0.997

Calves reacted significantly faster to Own cow playbacks (their own mothers) than to Other cows (Fig. 3.7; Wilcoxon signed-rank test: Z = -3.063, p = 0.002). In contrast, there was no significant difference in the latency to react in cows listening to playbacks of calls from Other and Own calves (Wilcoxon signed-rank test: Z = -1.858, p = 0.063).

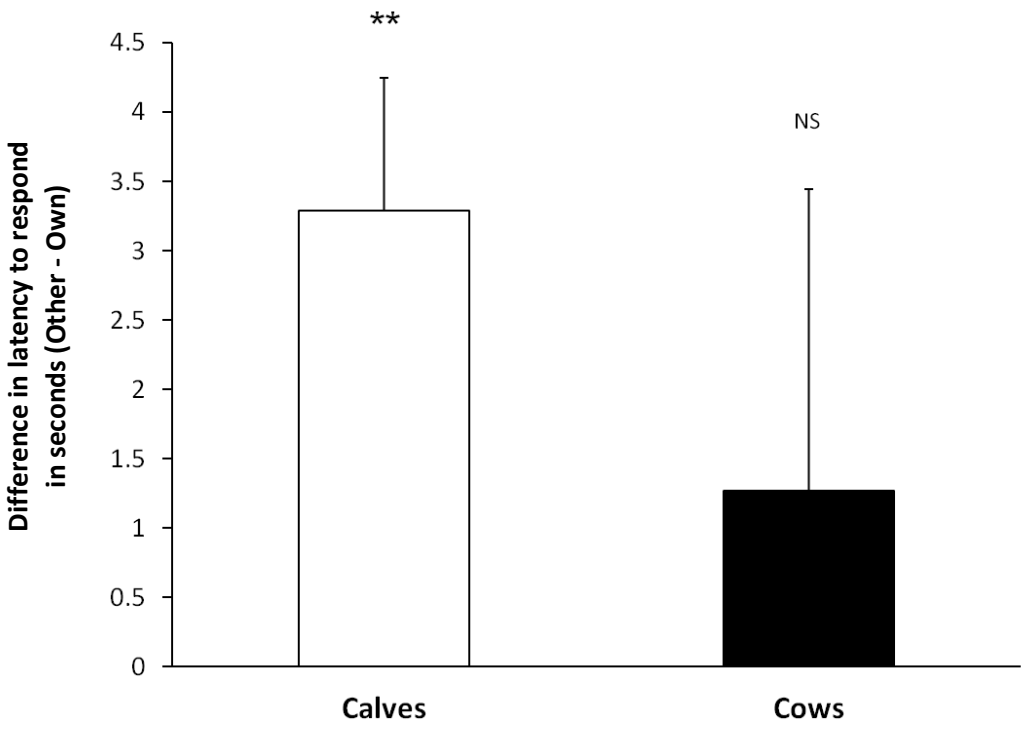

Figure 3.7. Average differences (+/- SEM) in the latency to respond to playbacks of calls from Own and Other animals in calves and cows (Wilcoxon signed-rank test: **p < 0.01, NS = non significant).

3.5 Discussion

Using playback experiments, I investigated if mother-offspring individual vocal recognition occurs in cattle. The ability of mother and offspring to identify each other (unidirectional vs. bidirectional recognition) is thought to be linked to predator avoidance strategies (hider vs. follower) in ungulates. The general consensus is that cattle is a hider species, and it is therefore predicted that unidirectional vocal recognition will be observed between cows and calves. However, contrary to this prediction, I found that mother-offspring individual recognition in cattle is a bidirectional process. The results support previous studies (Barfield et al. 1994; Marchant-Forde et al. 2002), which suggested that calves can distinguish the calls of their own mothers from those of other cows. More importantly, and for the first time, the results also show that cows are able to recognise the calls of their own calves. The presence of bidirectional parent-offspring individual recognition of vocalisations in cattle is not consistent with assumptions that cattle evolved to hide their young when separated from them during foraging, and that this "hider" strategy favours the evolution of unidirectional individual recognition in ungulates (Torriani et al. 2006; Sèbe et al. 2007).

Despite of the classification of domestic cattle as a hider species (Langbein & Raasch 2000; Flower & Weary 2003), and the prediction that hider species would show unidirectional recognition between offspring and mothers (Fisher

et al. 2002; Torriani et al. 2006; Briefer & McElligott 2011), the results of this study have revealed that cows and calves display bidirectional individual recognition. This important finding could reflect the fact that hiding behaviour in domestic cattle is relatively weak. The classification of domestic species as hiders or followers is not clear-cut because domestication involves animals being kept in artificial conditions in which their behaviour may be markedly constrained. Nevertheless, domestic cattle have commonly been classified as hider species because, although cattle in modern agricultural environments often do not have the opportunity to hide their young, when cover is provided, hiding behaviour has been observed (Langbein & Raasch 2000). Hiding behaviour may cease earlier in cattle than in other hider species. Le Neindre (1984) observed that calves had no neighbour in 12 % of the scans made when they were between 2 to 5 days old. This period of hiding is rather short, and three weeks after birth, calves spend most of their time with other calves. The fact that calves seem to display following behaviour relatively soon after birth, and the social integration with other conspecifics which this entails, might mean that selection has favoured bidirectional recognition. Similarly, domestic goats (*Capra hircus*), in which bidirectional acoustical recognition has also been observed (Briefer & McElligott, 2011), have been classified as a hider species, despite the fact that some researchers have reported that they do not display hiding behaviour under some domestic settings (Rudge 1970; Tennessen & Hudson 1981). Lickliter (1984) showed that rather than losing the hiding behaviour due to domestication process,

goats display a very short period of hiding (4 – 7 days after birth), as long as they have access to places to hide. Again, the relative weakness of this hiding behaviour, being followed by social integration, could be the reason why goats evolved bidirectional recognition.

Given the complex cognitive processes involved in bidirectional vocal recognition, it seems reasonable to assume that the ability of mothers and offspring to recognise each other observed today has evolved over a long period of time, and hence was present in ancestral wild cattle. Regardless of whether modern domestic cattle display hider behaviour, the existence of bidirectional recognition in domestic cattle could therefore be explained if a follower strategy or a weak hider behaviour was often adopted in wild ancestors of modern breeds. Unfortunately, it is not possible to know how the ancestral cattle behaved, but we can get clues from feral populations of ancient cattle breeds and other closely related bovid species. Nowadays, there are just a few feral cattle populations left that have been free from human management for a long time. The two best examples are Chillingham cattle, which have been classified as hider species (Hall 1986), and Maremma cattle, which have been observed displaying both hider and follower strategies in the early weeks of life, depending on the availability of cover (Vitale et al. 1986). Among the examples of other bovids studied, the American bison (*Bison bison*) and wildebeest (*Connochaetes taurinus*) have both been classified as followers according to behaviour observed in wild

populations (Estes & Estes 1979; Green 1992). Similarly, the European bison (*Bison bonasus*) although being a forest species, exhibit a following type of strategy for offspring protection, typical for ungulates living in open areas, no hiding phase was observed (Daleszczyk 2005). Given this variation in strategy seen in other bovids, and in feral breeds, it is not so straightforward to predict whether recognition between cows and calves should be uni- or bidirectional. It is plausible that ancestral cattle evolved bidirectional acoustic recognition under selection associated with a short hiding phase proceeded by a very early following behaviour, or facultative following behaviour expressed in relatively open habitats. Alternatively, bidirectional recognition could be a recent adaptation to the modern farm environment in which hiding is rarely possible. It is possible then to explain the mismatch between the patterns observed in this study and previous predictions about cattle behaviour. Indeed, it may be more generally true that attempts to divide ungulates into hiders and followers, and to make predictions about mother-offspring recognition based on this dichotomy without considering intermediate behavioural patterns (Ralls et al. 1986), are flawed.

Extensive research about maternal behaviour in captive ungulates (Ralls et al. 1986, 1987) has led to the conclusion that the hider-follower dichotomy is an overly simplistic characterization of the mother-offspring predator avoiding strategy which is not effective in describing the whole range of behavioural patterns adopted by ungulates. Ralls et al. (1986, 1987) have proposed that

many species in captivity (e.g. zebra (*Equus burchelli*), tapir (*Tapirus terrestris*), hippopotamus (*Hippopotamus amphibius*), camel (*Camelus bactrianus*), reindeer (*Rangifer tarandus*), giraffe (*Giraffa camelopardalis*), bison (*Bison bison*), wildebeest (*Connochaetes taurinus*), and gazelle (*Dorcas gazelle*) could belong to an intermediate category between hider and follower. Although this work considered captive animals, which often experience a very different environment from those experienced by wild ancestral ungulates, it may be more generally true that the hider-follower dichotomy may not actually be very helpful in understanding the evolution of the ability to recognise closely related individuals in ungulates.

Irrespective of the debate about the hider/follower dichotomy, when considering the relationship between the degree of detectable acoustic individuality seen in a species, and the behavioural strategies exhibited by that species in its evolutionary past, it is important to remember that detectable individuality does not necessarily need to "evolve" as an adaptive trait at all. Some degree of individuality must exist in all species which vocalise, as a necessary consequence of the unique combination of genotype and environment experienced by each individual. These combinations will generate differences among individuals in vocal-tract morphology, and hence in the acoustic properties of vocalisations. Similarly, the ability to detect individuality in conspecifics may arise as an inevitable consequence of selection on sensory and cognitive capabilities caused by the benefits of being

able to interpret other subtle differences in sounds in the environment. Hence, it may be the case that my discovery of marked individuality in cattle vocalisations (Chapter Two), and bidirectional recognition, implies nothing about the selective pressures associated with the behavioural strategy employed by mothers and offspring in the ancestors of modern cattle. Especially in the domestic environment, where population densities are high, latent variation in the acoustic properties of individual calls, combined with the generally sophisticated cognition typical of "higher" vertebrates, may allow cattle to learn to recognise the calls of specific individuals without this representing a specific adaptation to a particular problem posed by the ancestral environment.

My results show that the age of the calf is an important factor in determining a cow's response to playbacks. Specifically, mothers of younger calves tended to respond relatively more strongly than mothers of older calves to playbacks. These findings are similar to previous reports about the mother-offspring bond, where it has been shown that cows are more attentive for nursing, and in general express more maternal behaviour towards younger calves (Thomas et al. 2001; Keyserlingk & Weary 2007). It has been observed that the mother-offspring relationship diminishes over time as the calf grows and becomes more independent in modern domestic cattle (Thomas et al. 2001; Keyserlingk & Weary 2007), wildebeest (Estes & Estes 1979), American bison (Green 1992) and Maremma cattle (Vitale et al. 1986). In contrast, however, even though a

decrease in responsiveness in older calves might be expected as they become more independent (Thomas et al. 2001; Keyserlingk & Weary 2007; Estes & Estes 1979; Green 1992; Vitale et al. 1986), there was no reciprocal tendency in this study for older calves to pay less attention to playbacks of the calls of their mothers.

The results obtained in this study are consistent with previous studies where calves have been shown to be able to identify their own mothers from their calls (Barfield et al. 1994; Marchant-Forde et al. 2002). Interestingly, in my experiments, calves were generally less responsive to playbacks than cows, and only the weakest behavioural reaction (ear movement/looking at the speaker) in calves was significantly different in response to playbacks of calls from Own and Other cows. This could be explained by my observation that calves, once fed, would typically spend up to six hours without needing to suckle or be in contact with the mother. The playback trials were made opportunistically in the field, and calves were not therefore always hungry. If calves are motivated mostly by hunger, while mothers are perhaps more motivated by the risk of losing their calves, or possible predation risk, this could explain why calves were generally less likely to reply to their mother's calls.

It is important to note that the methodology used in the present study differs substantially from that used in previous studies (Barfield et al. 1994;

Marchant-Forde et al. 2002). My approach exposed animals to playbacks in conditions which resembled as closely as possible those likely to be encountered by a wild population. Free range cattle were chosen for the experiments, and they were observed in relatively unconstrained conditions in the field. The two observers tried to remain out of sight of the herd, and the equipment remained hidden in a camouflage tent (loudspeaker) and camouflage net (camera), which were positioned well in advance (between 1 to 2 hrs) before the start of each trial. Furthermore, in the present study cows and calves were never separated or under any kind of stress associated with artificial human manipulation. This contrasts with the previous playback studies (Barfield et al. 1994; Marchant-Forde et al. 2002), where calves were artificially separated from their mothers before each playback trial, and kept in an isolation pen. Such an approach might produce unrealistic results since behavioural and acoustic changes have been reported in cattle under the stress of isolation. For example, it has been widely demonstrated that during the weaning process, during which cows and calves are kept apart by farmers, both mothers and offspring vocalise more, have higher activity levels and place their heads outside of the pen more often (Lidfords 1996; Weary and Chua 2000; Flower and Weary 2001; Manteuffel et al. 2004). Additionally, cows separated from their calves after birth call with a higher fundamental frequency (Weary and Chua 2000).

3.6 Conclusion

In summary, my findings strongly suggest that individual vocal recognition between domestic cows and calves is bidirectional, and at least partly influenced by calf age. In order to understand how and why we see this pattern in a domestic setting, we need a greater understanding of the conditions under which individual recognition has evolved. In particular, we may need to move beyond the simple classification of species as “hider” or “follower”. Detailed comparative behavioural studies of domestic, feral and wild ungulates are needed to determine the differences in parent-offspring interactions within and among species. Additionally, given the possible existence of plasticity in “hider”/”follower” behaviour, studies of the influence of recent environmental and perhaps genetic changes associated with domestication on mother-offspring behaviour are also needed. Such studies will not only be of relevance in seeking to understand the fundamental evolutionary biology of communication, but, given the significance of mother-offspring behaviour for animal welfare and domestic production, will also have considerable relevance in applied fields.

3.7 References

Alexander, G. 1977. Role of auditory and visual cues in mutual recognition between ewes and lambs in Merino sheep. Applied Animal Ethology 3, 65-81.

Allan, C. A., Hinch, G.N., Holst, P. J. 1991. Behaviour of parturient Australian bush goats. II. Spatial relationships and activity patterns. Applied Animal Behaviour Science 32, 65-74.

Arave, C. W., Albright, J. L. 1981. Cattle behaviour. Journal of Dairy Science 64, 1318-1329.

Balcombe, J.P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana.* Animal Behaviour 39, 960-966**.**

Barfield, C. H., Tang-Martinez, Z., Trainer, J.M. 1994. Domestic calves (*Bos Taurus*) recognize their own mothers by auditory cues. Ethology 97, 257-264.

Blumstein, D. T., Munos, O. 2005. Individual, age and sex-specific information is contained in yellow-bellied marmot alarm calls. Animal Behaviour 69, 353-361.

Boersma P, Weenink D. 2009. Praat: doing phonetics by computer. http:www.praat.org/

Briefer, E., McElligott, A. G. 2011. Mutual mother-offspring vocal recognition in an ungulate hider species (*Capra hircus*). Animal Cognition 14, 585-598.

Charrier, I., Mathevon, N. & Jouventin, P. 2002. How does a fur seal mother recognize the voice of their pup? An experimental study of *Arctocephalus tropicalis*. Journal of Experimental Biology 205, 603-622.

Charrier, I., Aubin, T., & Mathevon. 2010. Mother-calf vocal communication in Atlantic walrus: a first field experimental study. Animal cognition 13, 471-482.

Coulon, M., Deputte B.L., Heyman, Y., Richard, C., & Delatouch, L. 2007. Visual discrimination by heifers (*Bos taurus*) of their own species. Journal of Comparative psychology 121, 198-204.

Coulon, M., Deputte B.L., Heyman, Y., & Baudoin C. 2009. Individual recognition in domestic cattle (*Bos taurus*): Evidence from 2D- Images of heads from different breeds. PLoS One 4, 1-8.

Daleszczyk, K. 2005. Activity pattern of calf and its integration into herd in European bison, *Bison bonasus.* Folia Zoologica 54, 359-363.

Edwards, S. A., Broom, D. M. 1982. Behavioural interactions of dairy cows with their newborn calves and the effects of parity. Animal Behaviour 30, 525-535.

Espmark, Y. 1971. Individual recognition by voice in reindeer mother-young relationship. Behaviour 40, 295-301

Espmark, Y. 1974. Individual characteristics in the calls of reindeer calves. Behaviour 54, 50-59.

Estes, R. D., & Estes, R. K. 1979. The birth and survival of wildebeest calves. Zeitschrift für Tierpsychologie 50, 45-95.

Fisher, D. O., Blomberg, S. P., & Owens, P. F. 2002. Convergent maternal care strategies in ungulates and macropods. Evolution 56, 167-176.

Fischer, J., Hammerschmidt, K., Cheney, D. L., & Seyfarth, R. M. 2002. Acoustic features of male baboon loud calls: influences of context, age, and individuality. Journal of Acoustic Society of America 111, 1465-1474.

Flower, F. C., & Weary, D. M. 2001. Effects of early separation on the dairy cow and calf. II: Separation at 1 day and 2 weeks after birth. Applied Animal Behaviour Science 70, 275-284.

Flower, F. C., & Weary, D. M. 2003. The effects of early separation on the dairy cow and calf. Animal Welfare 12, 339-348.

Frommolt, K. H., Goltsman, M. E., & Macdonald, D. W. 2003. Barking foxes, *Alopex lagopus*: field experiments in individual recognition in a territorial mammal. Animal Behaviour 65, 509-518.

Green, W. C. H. 1992. The development of independence in bison: pre-weaning spatial relations between mothers and calves. Animal Behaviour 43, 759-773.

Hall, S. J. G. 1986. Chillingham cattle: dominance and affinities and access to supplementary food. Ethology 71, 201-215.

Hall, S.J.G., Vince, M., Shillito, E. & Garson, P.J. 1988. Vocalisations of the Chillingham cattle. Behaviour 104, 78-104.

Jensen, P. 2001. Parental behaviour. In: Keeling, L. J., & Gonyou, H. W. (Eds.) Social Behaviour in farm animals, CABI Publishing, UK, pp. 59-81.

Keyserlingk, M.A.G., & Weary, D.M. 2007. Maternal behaviour in cattle. Hormones and Behaviour 52, 106-113.

Kroodsma, D. E., Byers, B.E., Goodale, E., Johnson, S & Liu W.C.. 2001. Pseudoreplication in playback experiments, revisited a decade later. Animal Behaviour 61, 1029-1033.

Langbein, J. & Raasch, M. L. 2000. Investigations on the hiding behaviour of calves at pasture. Archives für Tierzucht-Archives of Animal Breeding 43, 203-210.

Le Neindre, P. and Sourd, C. 1984. Influence of rearing conditions on subsequent social behaviour of Friesian and Salers heifers from birth to six months age. Applied Animal Behaviour Science 12, 43-52.

Lent, P. C. 1974. Mother-infant relationships in ungulates. In: Geist, V., & Walther, F. (Ed). Behaviour of ungulates and its relation to management. IUCN Publications, New Series 24. IUCN, Morges, Switzerland. pp. 14-55.

Lickliter, R. 1984. Hiding behaviour in domestic goat kids. Applied Animal Behaviour Science 12, 245-251.

Lickliter, R., & Heron J. R. 1984. Recognition of mother by newborn goeats. Applied Animal Behaviour Science 12, 187-192.

Lickliter, R. & Ness, J. 1990. Domestication and comparative psychology: status and strategy. Journal of Comparative Psychology 104**,** 211-218.

Lidfords, L. M. 1996. Behavioural effects of separating the dairy calf immediately or 4 days post-partum. Applied Animal Behaviour Science 49, 269-283.

Manteuffel, G., Puppe B., & Schön, P. C. 2004. Vocalizations of farm animals as a measure of welfare. Applied Animal Behaviour Science 88**,** 163-182.

Marchant-Forde, J. N., Marchant-Forde, R. M. & Weary, D.M. 2002. Responses of dairy cows and calves to each other´s vocalisations after early separation. Applied Animal Behaviour Science 78, 19-28.

McDougall, P. 1975. The feral goats of Kielderhead Moor. Journal of Zoology 176, 215-246

McElligott, A. G., Birrer, M., & Vannoni, E. 2006. Retraction of the mobile descended larynx during groaning enables fallow bucks (*Dama dama*) to lower their formant frequencies. Journal of Zoology 270, 340-345.

Nowak, R., Porter, R., Lévy, F., Orgeur, P., & Schaal., B. 2000. Role of mother-young interactions in the survival of offspring in domestic mammals. Reviews of Reproduction 5, 153-163.

Pitcher, B.J., Harcourt R.G., & Charrier, I. 2010. Rapid onset of maternal vocal recognition in a colonially breeding mammal, the Australian sea lion. PLoS ONE 5**,** 1-7.

R Development Core Team 2009. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org.

Ralls, K., Kranz, K., & Lundrigan B. 1986. Mother-offspring relationships in captive ungulates: variability and clustering. Animal Behaviour 34, 134- 145.

Ralls, K., Kranz, K., & Lundrigan B. 1987. Mother-Young relationships in captive ungulates: spacial and temporal patterns. Zoo Biology 6, 11-20.

Reby, D. & McComb, K. 2003. Anatomical constrains generate honesty: acoustic cues to age and weight in the roars of red deer stags. Animal Behaviour 65, 519-530.

Rendall, D., Rodman, P. S., & Emond, R.E. 1996. Vocal recognition of individuals, and kin in free-ranging rhesus monkeys. Animal Behaviour 51, 1007-1015.

Riede, T., Bronson, E., Hatzikirou, H., & Züberbühler, K. 2005. Vocal production mechanisms in a non-human primate: morphological data and a model. Journal of Human Evolution 48, 85-96.

Rudge, M.S. 1970Mother and kid behaviour in feral goats. Zeitschrift für Tierpsychologie 27, 687-692.

Searby, A. & Jouventin P. 2003. Mother-lamb acoustic recognition in sheep: a frequency coding. Proceedings of the Royal Society B 270, 1765-1771.

Sebe, F., Nowak, R., Poindron, P., & Aubin T. 2007. Establishment of vocal communication and discrimination between ewes and their lamb in the first two days after parturition. Developmental Psychobiology 49,375-386.

Stehulova, I., Lidfors, L., & Spinka, M. 2008. Response of dairy cows and calves to early separation: Effect of calf age and visual and auditory contact after separation. Applied Animal Behaviour Science 110, 144-165.

Tennessen, T., & Hudson,R. R. 1981. Traits relevant to the domestication of herbivores. Applied Animal Ethology 7, 87-102.

Thomas, T. J., Weary, D. M., Appleby, M. C. 2001. Newborn and 5-week-old calves vocalize in response to milk deprivation. Applied Animal Behaviour Science 74, 165-173.

Tibbets, E. A., & Dale, J. 2007. Individual recognition: it is good to be different. Trends in Ecology and Evolution 22, 529-537.

Torriani, M. V. G., Vannoni, E. & McElligott, A. G. 2006. Mother-young recognition in an ungulate hider species: a unidirectional process. American Naturalist 168, 412-420.

Tucker, C.B. 2009. Behaviour of cattle. In: Jensen, P. (Ed). The Ethology of domestic animals. 2nd edition. CABI, UK. pp. 151-160.

Trivers, R. L. 1972. Parental investment and sexual selection. In: Campbell, B. (Ed). Sexual selection and the descent of man*, 1871-1971.* Aldine-Atherton, Chicago, pp. 136-179.

Vannoni, E., Torriani M. V. G. & McElligott A. G. 2005. Acoustic signalling in cervids: a methodological approach for measuring vocal communication in fallow deer. Cognition, Brain, Behaviour 9, 551-565.

Vaughan, T. A., Ryan, J. M., & Czaplewski N.J. 2000. Behaviour. Mammalogy. 4th ed. Thompson learning, U.S.A.

Vitale, A. F., Tenucci, M., Papini, M., & Lovari, S. 1986. Social behaviour of the calves semi-wild Maremma cattle, *Bos primigenius taurus*. Applied Animal Behaviour Science 16, 217-231.

Watts, J. M., Stookey, J. M. 2000. Vocal behaviour in cattle: the animal's commentary on its biological processes and welfare. Applied Animal Behaviour Science 67, 15-33.

Weary, D. M., & Chua, B. 2000. Effects of early separation of the dairy cow and calf. 1. Separation at 6 h, 1 day and 4 days after birth. Applied Animal Behaviour Science 69, 177-188.

CHAPTER FOUR

Cattle behavioural responses to familiar and unfamiliar stimuli

4. Cattle behavioural response to familiar and unfamiliar stimuli

4.1 Introduction

Individual differences in behaviour within populations have been the focus of intense research interest over the past decade (Bell 2007; Stamps & Groothuis 2010; Wolf & Weissing 2012). The increasing interest results from the realisation that individual behavioural differences can be both stable over time and correlated across different contexts (Sih et al. 2003; Bell 2007), and that such differences are a common feature of animal populations, occurring in a diverse range of species across the animal kingdom (Sih et al. 2003; Rèale et al. 2007). Research in this area aims to understand the development, causation, evolution and function of behavioural individuality (Stamps & Groothuis 2010; Wolf & Weissing 2012).

Cattle are a highly social species with well-developed dominance relationships (Bouissou 1972; Albright & Arave 1997; Bouissou et al. 2001), and in recent years applied research has shown that individual differences in behaviour amongst cattle may have an important role to play in responses to handling and housing systems (Broom 1988; Manteca & Deag 1993), and consequently in productivity (Hemsworth et al. 2000). In this study, I aimed to look beyond situations in which cattle interact with humans, to explore the extent to which

cattle behavioural responses are individually consistent when exposed to novel stimuli in a semi-natural environment.

4.1.1 Individual behavioural differences

It has long been recognised that individuals of the same size, sex and from the same population frequently behave in different ways (Clark & Ehlinger 1987; Wilson 1998; Bell 2007). Nevertheless, the explicit study of individual differences is a relatively new field and there is still some confusion and controversy over terminology and definitions of the different kinds of individuality encountered in animal behaviour. "Animal personality", perhaps the most commonly identified type of behavioural individuality, is defined as consistent differences among individuals in a particular type of behaviour across time and contexts (Dingemanse et al. 2010; Rèale et al. 2007). Similarly, consistent differences which are repeatable though time are considered "personality traits" (Stamps 2007; Dingemanse et al. 2010; Carter et al. 2012). “Temperament” and “coping styles” are terms that initially developed in separate literatures, but their meaning for behavioural science has recently converged with the definition of "personality" (Rèale et al. 2007; Stamps & Goothuis 2010). Recently, the term "behavioural syndrome" was introduced, and is defined as suite of correlated behaviours or traits that reflect between within-individual consistency in behaviour across time and multiple situations or contexts (e.g. aggressiveness-boldness; Sih 2004; Bell 2007; Sih et al. 2012;

Wolf & Weissing 2012). A population or species can exhibit a behavioural syndrome. However, within the syndrome, individuals have a "behavioural type" or "personality trait" (e.g. a more aggressive type or a less aggressive type; Sih 2004; Bell 2007). Nevertheless, somewhat confusingly, many researchers studying this field consider that "personality", "coping styles" "temperament" are all analogous to the term "behavioural syndrome" (Sih 2004; Bell 2007; Rèale 2007; Sih et al. 2012). Others have stated that any pattern of behaviour which satisfies the criteria for personality also satisfies the criteria for behavioural syndrome, but that the reverse is not the case (Stamps & Groothuis 2010; Wolf & Weissing 2012). For the purposes of this study, I will try to avoid the controversy over these different interpretations of the terminology. For consistency with other studies of cattle behaviour (see below), I will use the term “temperament” to describe consistent differences in behaviour among individuals in my study population through time and across contexts.

4.1.2 Boldness and shyness

Individual differences in personality traits have been studied in many animals such as fish (e.g. Wilson et al. 1993; Harcourt et al. 2009; Wilson & Godin 2009), amphibians (e.g. Sih & Watters 2005), reptiles (e.g. Riechert & Hedrick 1993), birds (e.g. Carere et al. 2005), and mammals (e.g. Carter et al. 2012; Rèale, 2007). Many different personality traits have been identified, such as

boldness, aggressiveness, sociability, activity, exploration, shyness, etc. For the purposes of the present study I will focus primarily on boldness and shyness. Boldness, which has been called both a behavioural trait (Mathot et al. 2012) and a personality type (Sih et al. 2012), can be defined as the tendency of individuals to be exploratory and take risks, particularly in novel contexts (Wilson et al. 1994; Atwell et al. 2012).

The shy-bold continuum is likely to be a common phenomenon in natural populations and to be widely-distributed taxonomically (Wilson et al 1994). Behavioural types (e.g. bold and shy) can affect the fitness of an individual (Smith & Blumstein 2008, Biro & Stamps 2008). When different behavioural types are clearly favoured in different environments, individual variation can result in suboptimal behaviour in some environments. For example, individuals that exhibit a bold behavioural type can sometimes take unnecessary risks and therefore suffer high mortality in dangerous environments (Carter et al. 2010), whereas more cautions individuals may miss opportunities to access resources in safer situations (Sih et al. 2003; Sih et al. 2012).

4.1.3 Individual behaviour in cattle

Individual differences in cattle behavioural activity are conspicuous during normal farming activities (Muller & Schrader 2005a), and also in response to

challenging situations, such as social separation (Muller & Schrader 2005b). Because most of the studies concerning individual behavioural differences in domestic or farm animals stem from the consequences of such differences for the handling and management of animals by farmers, there is a tendency to use the term "temperament" instead of personality. I will thus henceforth use “temperament” to refer any consistent differences among individuals in their behaviour across different contexts (Dingemanse et al. 2010; Rèale et al. 2007).

Temperament has an important effect on cattle responses to human handling (Burrow 1997), and as a result a number of diagnostic tests have been developed to help farmers characterise the temperament of their animals. However, to date there is no real consensus about a definitive set of criteria for the assessment of temperament. For example, the flight speed (FS) test measures the time that it takes an animal to cover a set distance after leaving a confined area (Burrow & Dillon 1997). Animals that cover the distance faster are considered to have a “poor” temperament, and individuals with slow FS scores are considered to have a "good” temperament (Burrow & Dillon 1997; Patherick et al. 2002). FS scores correlate negatively with weight gain in cattle, and hence have potential as indicators of productivity (Fordyce & Goddard 1984; Burrow & Dillon 1997). However, their behavioural significance remains unclear. Although it has been hypothesized that FS scores may measure innate fearfulness or shyness in regard to human handling (Patherick et al.

2002), other researchers have found that animals with high FS scores are not necessarily are less active, social or explorative. Thus, the classification of an animal's temperament based solely on its FS is not really appropriate (Müller & von Keyserlingk 2006).

Fear-related behaviours have received considerable attention in the applied literature on cattle behaviour. Reactions to stimuli indicating potential threats (e.g. those associated with predators) in farm animals might be used to predict an individual's ability to adapt to the constraints of husbandry, and thus to improve the efficiency of production and possibly the welfare (Boissy & Bouissou 1995). In order to characterise behavioural responses by cattle to threats, experiments have been conducted using stimuli from dogs and unfamiliar humans (Welp et al. 2004), wolves (*Canis lupus*) and mountain lions (*Puma concolor*) (Kluever et al. 2009; Laporte et al. 2010). Results show that cattle tend to modify their behaviour upon detection of a potential threat, increasing vigilance and keeping close to conspecifics. Most of these studies have not, however, established whether such reactions vary significantly and consistently among individuals, or whether they correlate with other aspects of temperament. Their ability to inform us about individuality in cattle behaviour is also limited by the conditions in which the experiments were conducted: animals were typically confined and socially isolated (and hence probably under considerable stress).

Reactions of cattle to novel objects have also been examined in some studies (Herskin et al. 2004; Kilgour et al. 2006; Gibbons et al. 2009). Results show, for example, that dairy cows show increased behavioural responses characterized by increased exploration, arousal and behavioural conflict when exposed to novel food or an unfamiliar person compared with inanimate novel objects (Herskin et al. 2004). Again, however, these studies did not quantify variation among individuals, or the consistency of that variation.

Although previous research into temperament in cattle has had a primarily economic motivation, the study of individual differences is also relevant to animal welfare. The welfare of an individual depends on whether it can cope with environmental challenge (Broom 1988; Manteca & Deag 1993; Boissy & Bouissou 1995), and this is highly likely to be influenced by behavioural individuality. A fuller understanding of temperament will thus help us to design better facilities and practices for managing animals in the farm environment. Furthermore, since better welfare means healthier, less stressed animals with more predictable behaviour, an understanding of temperament has the potential to provide benefits in terms of safety for human handlers and cattle themselves (see for example Le Neindre el al. 1995).

4.1.4 Heterospecific recognition in cattle

Consistent differences in behaviour among individuals take on added significance if individuals are able to identify one another accurately, since behavioural variance can play a key role in determining the nature and stability of social groups (Drews 1993). Recent research has highlighted the adaptive significance of the ability to identify not just conspecifics (Hagen & Broom 2003), but also individuals of different species (Kluever et al. 2009). Accurate identification of heterospecific individuals associated with particular rewards or threats can allow animals to fine-tune their behaviour to maximise fitness. Such fine-tuning may be especially conspicuous in domestic animals, where specific individual humans and those of other species (e.g. dogs) are consistently associated with either resources (e.g. farmers with food) or danger (e.g. stray dogs which are liable to attack).

It is known that adult cattle are able to identify conspecifics and even different breeds efficiently only by visual discrimination. Using an experimental design with 2D images from cow breeds with different coat patterns, it has been shown that cattle use visual discrimination in coat patterns, and familiarity improves their performance in recognition (Coulon et al. 2007 & 2009). However, no research has been done in cattle on acoustic

recognition either of conspecifics (except mother-offspring recognition studies, see Chapter 3) or heterospecifics.

4.2 Objectives

Most previous studies in cattle of temperament in general, and in particular of boldness and responses to threats, have been performed under intensive farm husbandry conditions. Here, I aimed to characterise exploratory and risk-averse behaviour and individuality in a free-range cattle herd in a semi-natural environment by measuring reactions to novel stimuli. I performed two experiments. In the first, animals were presented with three different novel objects whilst foraging unconstrained in a familiar environment. I looked for behavioural individuality in the tendencies of animals to approach and interact with these objects. The idea was to establish whether some individuals sit in different positions on a "boldness" – "shyness" continuum, where boldness is defined as the willingness to take risks in absence of a food reward (Kruvers et al. 2010). In the second experiment, individual behavioural responses to heterospecific playbacks of familiar and unfamiliar stimuli (humans, dogs and a pack of howling wolves) were assessed. Again, I looked for individuality in responses, indicative of variation among animals in the way that they cope with novel stimuli.

4.3 Methodology

4.3.1 Study site and subjects

Two experiments were carried out with two cattle herds (Herd 1: n = 21 adult females; n = 18 calves; n = 1 bull; Herd 2: n = 23 adult females; n = 13 calves; n = 1 bull) situated in two separated fields (52 ha and 23 ha, respectively) on a farm in Nottinghamshire, UK (52° 93´ 72", 1° 06´ 09´´W), from August of 2010 to December 2010. Calves included in this study were all born between February and August 2010, and all were sired by the same bull. All the calves included in the study were kept in the same field as their mothers all year long. All individuals included in the study were free to roam in the fields with fresh grass and water *ad libitum*. Accurate individual identification was obtained from specific ID tags placed in the animals' ears by the farmers, and by visual recognition of coat markings.

4.3.2 Experiment 1: Novel object presentation

The behavioural responses of individual cows and calves (n =26 adult females; n = 12 calves) to three different novel objects were considered in order to assess individual behaviour consistency and "boldness". Each experimental trial consisted of the presentation of one novel object to an identified individual in the herd. Three different objects, all the same colour, were used:

Figure 4.1. A calf (Athena) with the purple washing basket.

Figure 4.2. A cow (Bean) being presented with the purple bag.

Figure 4.3. A cow (Dark face) with the purple umbrella

a purple plastic washing basket, (46 cm diameter and 28.5 cm high; Figure 4.1), a purple square plastic bag (38 cm x 38 cm; Figure 4.2), and a purple umbrella (69 cm diameter; Figure 4.3). A total of 117 experimental trials were performed (three objects for each of 26 cows and 11 calves).

Objects were presented to randomly selected individuals in a random order. Each object was placed 1 and 3 m directly in front of the individual, always by the same experimenter. The experimenter walked in front of the animal, in plain sight, and placed the object on the ground, avoiding sudden movements, before walking away immediately. A second experimenter video-recorded the

trial with a digital video camera (Sony DCR-SR58), starting 1 min before the object was presented. The behavioural response was recorded for 5 min after the presentation of the object. After every presentation the objects were wiped with disinfectant solution to remove any odours associated with the previous individual tested.

4.3.3 Behavioural data

In order to assess the behavioural responses of cows and calves to the novel object presentation, I first inspected the videos, counting the number of times that a certain individual performed a particular behaviour (see Table 4.1 for a list of behaviours scored) towards the novel object during the 5 minutes after the presentation. The counts of behaviours were subject to Principal Components Analysis (PCA - see Section 4.4.1 below), and the extracted principal components were used to test for individuality in responses. This approach assumes that all observed behaviours were independent and have equal weight. In truth, it appeared in the field that the behaviours occurred in particular sequences, the end-point of which was indicative of the general strength of the positive or negative response by an animal to the objects. For example, the most positive reactions involved touching, licking or sniffing behaviours, which were normally preceded by looking and approaching in that order. Somewhat less positive sequences ended in approach behaviour, which was typically preceded by looking, but never by touching, licking or sniffing.

Apparently negative responses were more simply characterised by the animal walking or running away.

Table 4.1. Behaviours performed in response to novel object presentations. Behaviours were divided into those which appeared to reflect a positive or "bold" reaction, those which appeared to reflect a negative or "shy" reaction, and those for which the interpretation was ambiguous or neutral.

Positive behaviours	Neutral behaviours	Negative behaviours
Licking	Calling	Walking away
Sniffing	Ignoring	Running away
Touching		
Approaching		
Looking		

4.3.4 Statistical analysis

In the first analysis of behavioural responses to objects, PCA was used due to the possible intercorrelation of the assessed behavioural responses. The PCs with Eigen values greater than 1 were retained and were then used as input variables for the subsequent statistical analysis (IBM SPSS 2011). Differences among individuals, animal types, and treatments, were assessed using general linear mixed models (GLMMs), fitted using R Version 2.14.1. For each PC obtained, animal type (cow, calf) was fitted as a fixed factor, and animal ID and treatment (object type) were fitted as random factors. The significance of terms was tested with likelihood ratio (LR) tests following Zuur et al. (2009).

The random factor was tested by comparing models that were fitted with Restricted Maximum Likelihood (REML), and fixed factors were tested by comparing models fitted with Maximum Likelihood (ML).

4.3.5 Experiment 2: Playbacks of familiar and unfamiliar heterospecific vocalizations

To assess the individual responses of cattle to familiar and unfamiliar heterospecific vocalisations, pre-recorded vocalizations of humans, dogs and a group of grey wolves (*Canis lupus*) were played back to each of the two cattle herds being studied. Human and dog vocalizations were classified as familiar or unfamiliar depending on whether the cattle had previously encountered the individual that was the source of the recording. The two familiar humans were the owners of the herds: a 27 year old female called Catherine, and a 50 year old male called David. The two familiar dogs were a male Doberman (Hades) and a male German shepherd (Chino), both of which belonged to the farm and were observed to interact regularly with the herds. Two unfamiliar humans and two unfamiliar dogs which had never been encountered by the herds were selected which matched as closely as possible the sex, age, size and (in the case of the dogs) breed of the familiar individuals. Since wolves are extinct in the UK, the wolf calls used were unfamiliar to the cattle.

4.3.6 Sound recording and signal acquisition

Recordings of individual humans and dogs vocalizations were made in August of 2010 (summarised in Table 4.2). Human volunteers were asked to replicate the call that the owners of the herds use to attract the attention of their animals when providing winter food etc. ("come up"). The dogs were recorded barking and growling in aggressive contexts. Calls were recorded at distances of 2 - 3 m from the vocalizing human or dog with a Sennheiser MKH70 directional microphone, connected to a Marantz PMD660 digital recorder (sampling rate 44.1 kHz). Vocalizations were uploaded to a computer at a sampling rate of 44.1 kHz and saved in a WAV format at 16-bit amplitude resolution. I used Praat v.5.0.47 DSP Package (Boersma and Weenink, 2009) to build the sequences up for playback experiments.

Table 4.2. Summary of familiar and unfamiliar vocalizations used in this study.

Familiar vocalizations	Identity	Vocalizations	Unfamiliar vocalizations	Identity	Vocalizations
Humans	Catherine	"Come up" call	Humans	Heather	"Come up" call
	David	"Come up" call		Tom	"Come up" call
Dogs (*Canis domesticus*)	Hades (Doberman)	Aggressive barks/growling	Dogs	Doberman	Aggressive barks/growling
	Chino (German shepherd)	Aggressive barks/growling		German shepherd	Aggressive barks/growling
			Wolf (*Canis lupus*)	Unknown	Howling

Calls were individually visualized in spectrograms in Praat (FFT method, window length = 0.1 s, time steps = 100, frequency steps = 250, Gaussian window shape, dynamic range = 40 dB). All calls were subsequently inspected, and the highest quality recordings (where the call was clear, with little background noise) were used to construct sequences.

Call sequences from unfamiliar individuals were designed to match in structure sequences recorded opportunistically from familiar individuals. In the case of the humans, for Catherine (familiar) and Heather (unfamiliar), sequences consisted of a total of nine "come up" calls in 32 seconds, with ten seconds of silence before and after the sequence. For David (familiar) and Tom (unfamiliar), sequences consisted of a total of ten "come up" calls in 30 seconds, with ten seconds before and after the sequence.

Dog vocalizations were a mix of aggressive barking and growls recorded opportunistically during “natural” encounters with unfamiliar humans. The familiar dogs (Hades and Chino) were recorded while they were enclosed inside a car with the windows open and their owners outside. Sequences of vocalizations (a mixture of barks and growls), 34 sec in duration, were constructed, with ten seconds of silence before and after the sequence. The unfamiliar dogs were housed in the RSPCA Radcliffe Animal Shelter (32 Nottingham Road Radcliffe-on-Trent, Nottinghamshire, NG12 2DW). I

recorded their vocalizations in response to the presence of a stranger (myself) while they were enclosed in their individual housing. I then edited the recordings to create sequences which were a mixture of barks and growls, 34 sec in durations and with ten seconds of silence before and after. Finally, a sequence of 23.89 sec of a pack of wolves howling, with ten seconds of silence before and after, was obtained from a database of wolf vocalizations (Anonymous 2010; WolfCountry net: http://www.wolfcountry.net/WolfSounds.html).

4.3.7 Playbacks

All playback trials were performed opportunistically in the field, without any artificial isolation or manipulation of the animals, and while trying to cause the least disturbance possible. Nine different vocalization sequences were used (from four dogs, four humans and one pack of wolves), with each being played back three times giving a total of 27 playback trials. I played back call sequences, stored as mp3 files on a CD at sampling rate of 44.1 KHz, using a Skytronic TEC076 portable speaker system (frequency response: 50-20 kHz ± 3dB). The loudspeaker was hidden with a camouflage tent or behind bushes, 10 – 30 m away from the animals being tested. I tried to avoid auditory and visual contact with the animals as much as possible before and during the playback. In each playback trial, the behavioural responses of as many individuals in the herd as possible were filmed simultaneously from two

different locations with digital video cameras (a Sony DCR-SR58 and a Panasonic SDH-H80) set up 5 – 20 m from the tested animals.

4.3.8 Behavioural responses

The behavioural responses of cows and calves were assessed from videos of the playbacks in two ways. In the first approach, the proportion of all individuals in the herds that were visible on camera during the playback which were seen to perform each behaviour was calculated (between 18 and 74 individuals; pooled across 27 playback trials). The following behaviours were scored: looking towards the loudspeaker, approaching the loudspeaker, calling back and moving away from the loudspeaker. In a second approach, the same behavioural responses were considered but in this case as many animals as possible were individually identified from distinguishing marks (n = 39 cows and n = 24 calves; pooled across 27 playback trials). Each identifiable individual in the herd was allocated a binary response score indicating if each behaviour was performed or not during the playback. Animals which could not be identified from videos were excluded from this second analysis.

4.3.9 Statistical analysis

The analysis of cattle responses to heterospecific audio playbacks was undertaken in two stages. In the first stage, I analysed the general behavioural

response of the whole herd to the audio stimuli in the playback experiments. PCA was used due to the possible intercorrelation of the assessed behavioural responses. The PCs with Eigen values greater than 1 were retained and were then used as input variables for the subsequent statistical analysis (IBM SPSS version 20, 2011). The effects of the familiarity and identity of the stimulus were assessed using general linear mixed models (GLMM's). All models were fitted using R version 2.15.1 (R Development Core Team 2009), and model simplification by stepwise deletion from a saturated model was employed to test the significance of each factor. For each PC obtained, the familiarity of the recording played back was fitted as a fixed factor, and the stimulus (specific identity of the recording being played back) was considered as a random factor. The significance of terms was tested with likelihood ratio (LR) tests following Zuur et al. (2009). The random factor was tested by comparing models that were fitted with Restricted Maximum Likelihood (REML), and the fixed factor was tested by comparing models fitted with Maximum Likelihood (ML).

In a second approach, in order to test for individual differences in responses to heterospecific audio playback stimuli, generalised linear mixed models (GLMMs) with binomial errors were fitted for each of the three most common responses (look, approach and call). Moving away was not considered in the analysis because very few individually identifiable animals performed this behaviour during the trials. Models were fitted as above, with familiarity of

the stimuli (familiar and unfamiliar) and type of animal (cow or calf) as fixed factors, and individual cattle identity and playback trial as random factors. The inclusion of trial as a random factor was important because individual responses within a given trial were clearly correlated, presumably because animals reacted not only to the stimulus, but also to the reactions of other individuals to the stimulus

4.4 Results

4.4.1 Experiment 1: Novel object presentation

PCA of ten original behavioural variables (see Table 4.1) generated three PCs for all the behavioural responses assessed (n = 26 cows, n = 12 calves). Overall, these PCs explained 57.86 % of the variance in the behavioural responses analysed (Table 4.3). The first PC explained 27.58 % of the variance, the second PC explained 16.41 % of the variance, and the third PC explained 13.87 % of the variance. The first PC was strongly and positively correlated with all behavioural responses associated with boldness (positive responses = more willing to explore).

Table 4.3. Results of Principal Component Analysis (PCA) of behavioural responses towards novel objects. Bold type indicates loadings > 0.5.

	Component		
	PC1	PC2	PC3
Approaching	**0.790**	0.271	0.242
Backing away	-0.059	-0.408	**0.654**
Calling	0.244	**0.624**	0.107
Licking	**0.788**	-0.378	-0.237
Looking	0.452	-0.423	**0.551**
Runaway	0.264	-0.235	-0.266
Sniffing	**0.532**	**0.574**	0.007
Touching	**0.784**	-0.207	-0.257
Walking away	0.055	0.315	**0.503**

The second PC was positively associated with just two behavioural responses, calling and sniffing, which could be associated with boldness too. The third PC was positively correlated with behaviours related to shyness (negative responses = less willing to explore and/or more fearful).

There was a significant effect of animal type (cow versus calf) on PC1, with calves being more likely to display positive or bold responses to objects (GLMM: LR = 8.1760, df = 1, p = 0.0042; Figure 4.4). No significant random effects on PC1 of object type (GLMM: LR = 0.349, df = 1, p = 0.554) or individual identity were found (GLMM: LR = 0.735, df = 1, p = 0.391), suggesting that responses to the three different objects were uniform, and that any differences among animals were accounted for solely by their type.

There were no significant differences in PC2 between animal types (GLMM: LR = 2.250, df = 1, p = 0.133; Figure 4.4), and there were no significant random effects of object type (GLMM: LR = 0.311, df = 1, p = 0.577) or animal ID (GLMM: LR = 0.757, df = 1, p = 0.384.

There was a significant effect of animal type (cow versus calf) on PC3, with calves being more likely to display negative or shy behaviours (GLMM: LR = 5.0450, df = 1, p = 0.024; Figure 4.4). Again, no significant random effects on PC3 of object type (GLMM: LR = 0.4439, df = 1, p = 0.443) or animal ID (GLMM: LR = 5.476 e-08, df = 1, p = 0.999) were evident.

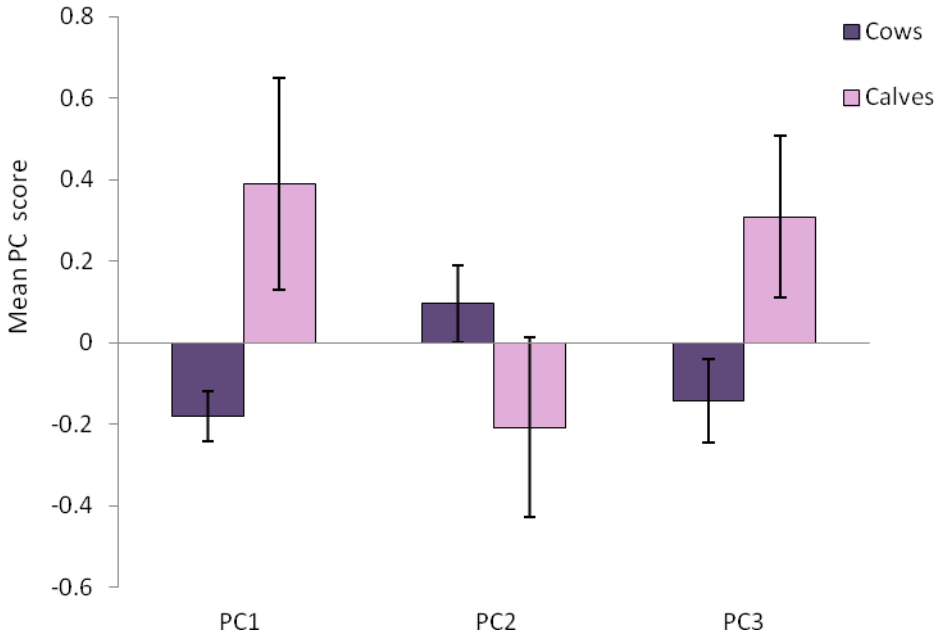

Figure 4.4. Effect of animal type (cow or calf) on PC1, which grouped the boldest behaviours; PC2 which grouped just calling and sniff; and PC3 which grouped the shyest behaviours. Data shown are the averaged PCs obtained for each type of animal (± SEM).

4.4.2 Experiment 2: Playbacks of familiar and unfamiliar vocalizations

Video recordings of the herds responding to playbacks of familiar and unfamiliar vocalizations captured behavioural responses of between 18 and 74 animals in each trial. PCA of four original behavioural variables characterising the response of the whole herd (the proportion looking, approaching, calling and moving away) generated 2 PCs, explaining 75.72 % of

the variance in the behavioural responses analysed (Table 4.4). The first PC explained 48.61 % of the variance, and the second PC 27.10 % of the variance. PC1 was strongly and positively correlated with all behaviours associated with boldness (positive responses = more willing to explore) after the heterospecific playback. In contrast, PC2 was strongly and positively associated with the unique behaviour associated with shyness (negative response = less willing to explore and/or more fearful).

Table 4.4. Results of Principal Component Analysis (PCA) of whole-herd behavioural responses to heterospecific audio playbacks. Bold types indicates loadings > 0.5.

	Component	
	PC1	PC2
Proportion looking	**0.922**	-0.045
Proportion calling	**0.634**	0.380
Proportion approaching	**0.828**	-0.329
Proportion moving away	0.079	**0.911**

No significant effects of familiarity (GLMM: LR = 0.600, df = 1, p = 0.439) or stimulus identity (LR = 1.145, df = 1, p = 0.285) on PC1 were found. Similarly, no significant effects of familiarity (LR = 0.491, df = 1, p = 0.483) or stimulus identity (LR = 0.370, df = 1, p = 0.543) on PC2 were found.

In the second stage of the analysis, where I examined the behavioural responses (looking, approaching and calling) of individually identifiable cattle

(n = 39 cows and n = 24 calves), significant individual differences in the tendency to "look" at the loudspeaker in response to the audio stimuli were detected (GLMM for ID: LR = 7.728, df = 1, p = 0.005; Figure 4.5). There was also a large significant random effect of trial on the tendency of cattle to look at the loudspeaker (LR = 93.94, df = 1, p < 0.001; Figure 4.6). No significant random effect of stimulus identity on looking behaviour were found (LR = 0.2362, df = 1, p = 0.627). Similarly, there were no significant fixed effects of type of animal (LR = 0.03165, df = 1, p = 0.5737) or familiarity (LR = 0.3671, df = 1, p = 0.5446), and no interaction between these factors (LR = 1.1182, df = 1, p = 0.2903).

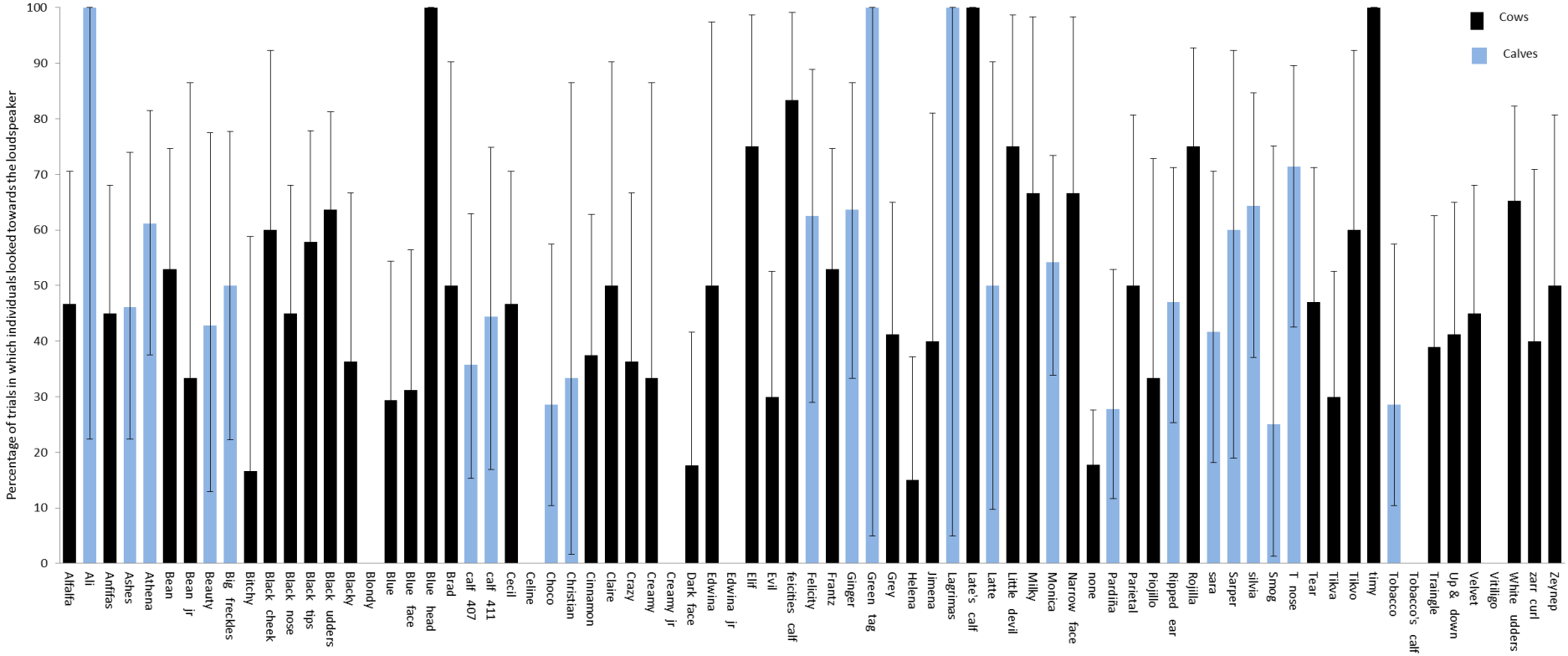

Figure 4.5. Percentage of trials in which individuals looked towards the loudspeaker after the heterospecific stimuli were played back (+/- 95 % confidence intervals based on the binomial distribution)

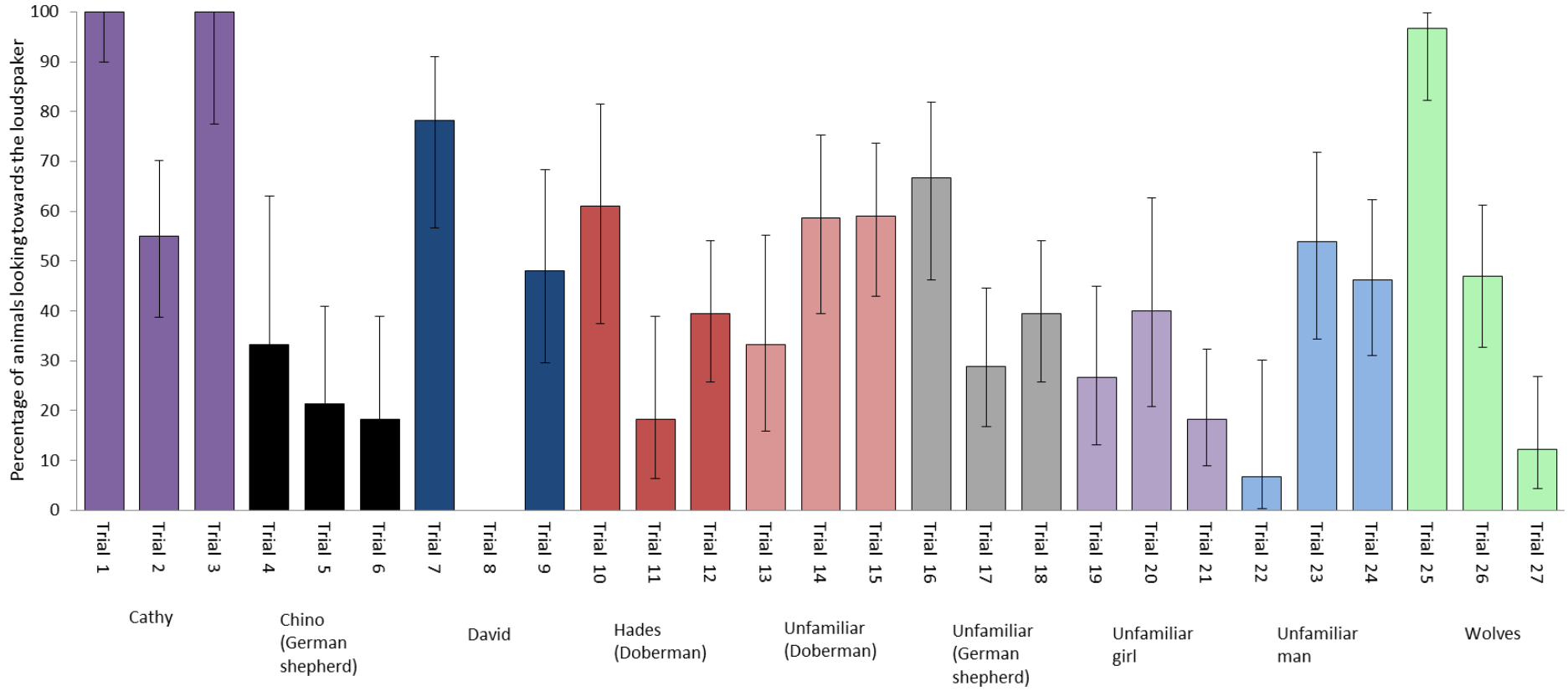

Figure 4.6. Percentage of animals looking towards the loudspeaker after each playback trial (+/- 95 % confidence intervals based on the binomial distribution).

Cattle also showed significant individual differences in the tendency to approach the speaker in response to the audio stimuli (GLMM for ID: LR = 17.951, df = 1, $p < 0.001$; Figure 4.7). Significant differences in the tendency to approach the speaker were also found among trials (LR = 179.25, df = 1, $p < 0.001$; Figure 4.8). No significant effect of stimulus identity on the tendency to approach the speaker was detected (LR = 0.0598, df = 1, p = 0.8068). Similarly, neither the type of animal (LR = 1.0084, df = 1, p = 0.3153), the familiarity of the stimulus (LR = 3.1019, df = 1, p = 0.0782), or the interaction between these two factors (LR = 0.4073, df = 1, p = 0.5234) had a significant effect on the tendency to approach the speaker.

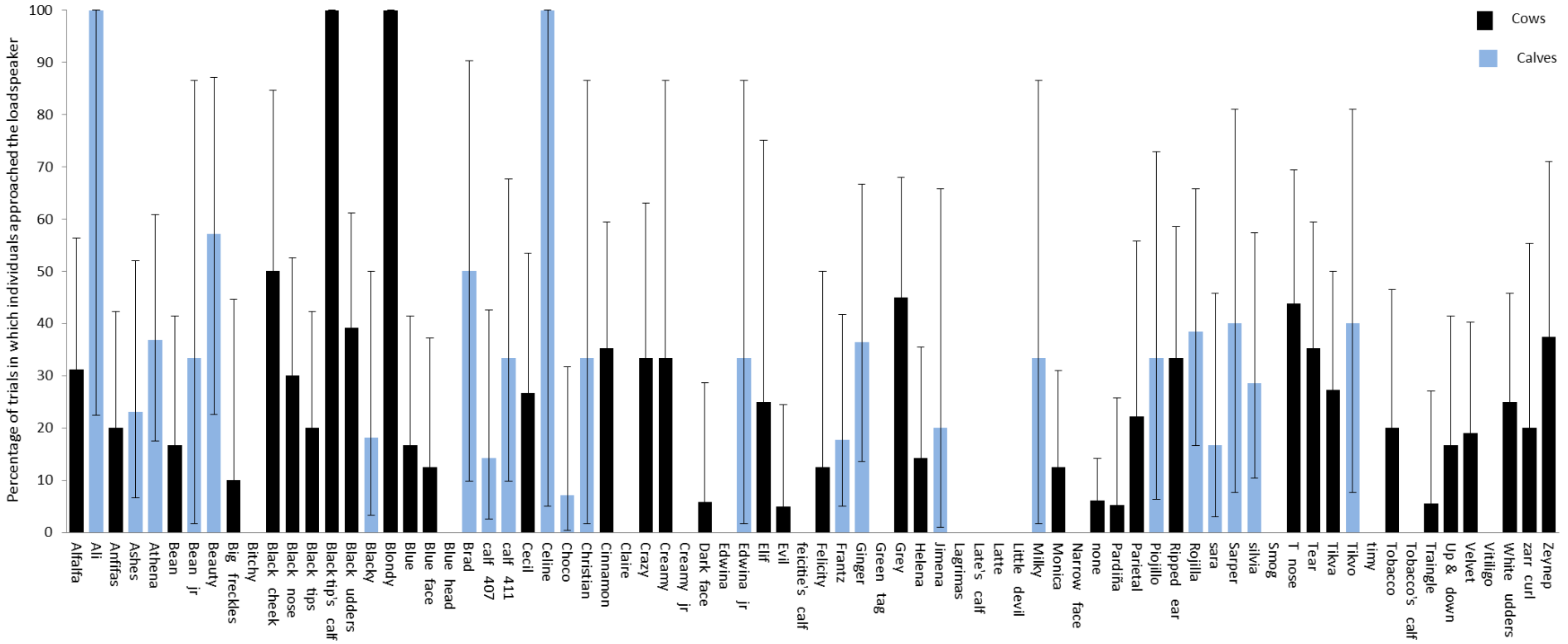

Figure 4.7. Percentage of trials in which individuals approached the loudspeaker after the heterospecific stimuli were played back (+/- 95 % confidence intervals based on the binomial distribution).

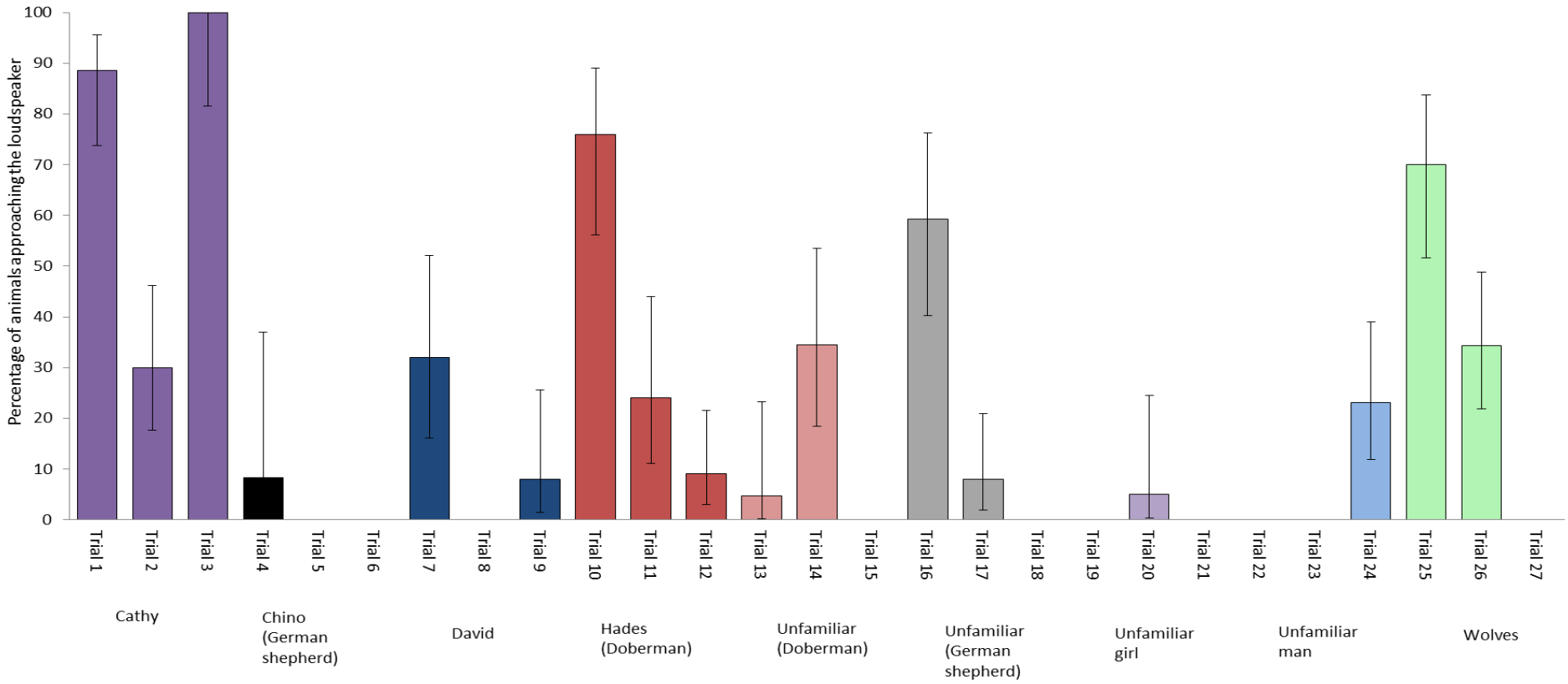

Figure 4.8. Percentage of animals approaching the loudspeaker after each playback trial (+/- 95 % confidence intervals based on the binomial distribution).

Cattle showed significant differences among trials in the tendency to call in response to the audio stimuli (LR = 4.8422, df = 1, p = 0.02777; Figure 4.9), but there were no significant differences among individuals (LR = 0.1772, df = 1, p = 0.6738) or among individual stimuli (LR = 0.0888, df = 1, p = 0.7657). Overall, cows were significant more likely than calves to call in response to playbacks (LR = 8.8428, df = 1, p = 0.002; Figure 4.10). There was no main effect of stimulus familiarity on the tendency to call (LR = 1.4814, df = 1, p = 0.2235), and although calves actually called more frequently after playbacks of unfamiliar heterospecifics (see Figure 4.10), the interaction between familiarity and animal type was not significant (LR = 2.917, df = 1, p = 0.08765).

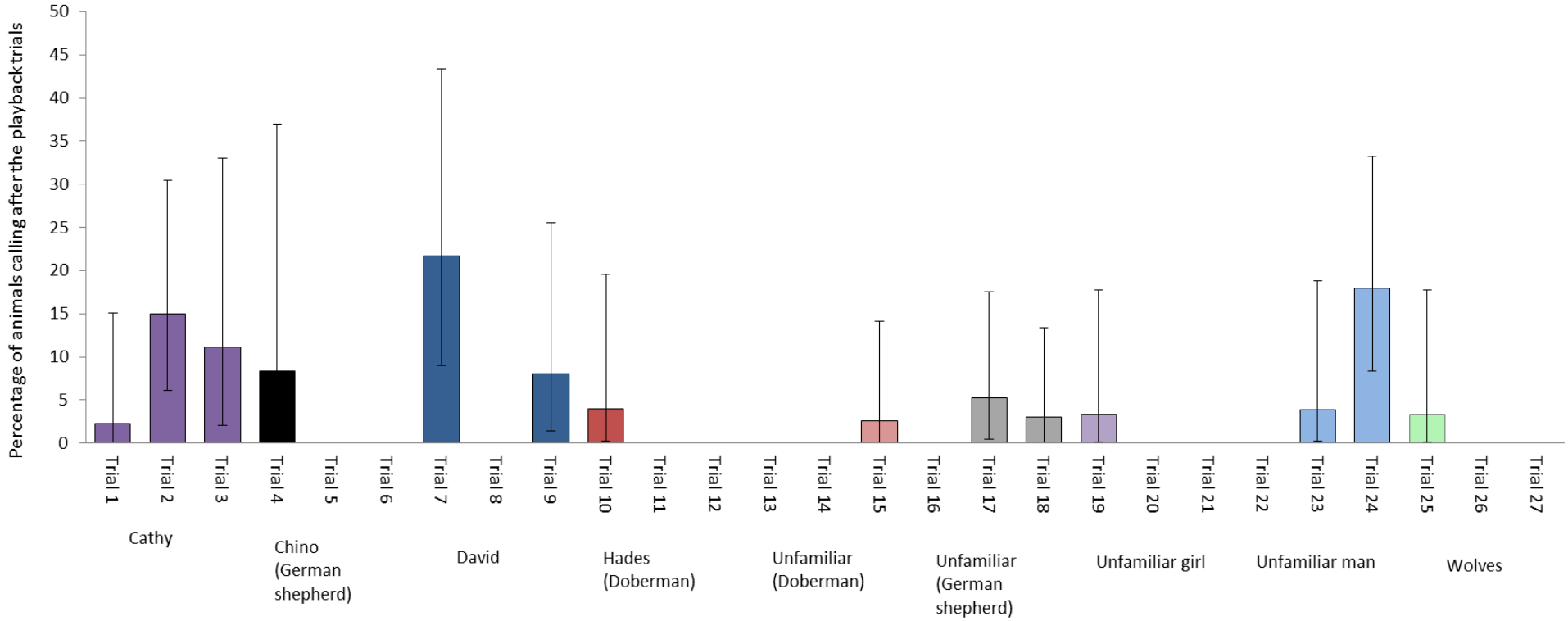

Figure 4.9. Percentage of animals calling after every playback trial (Chi-squared $_{(DF)}$ = 4.8422$_{(1)}$, p = 0.02777).

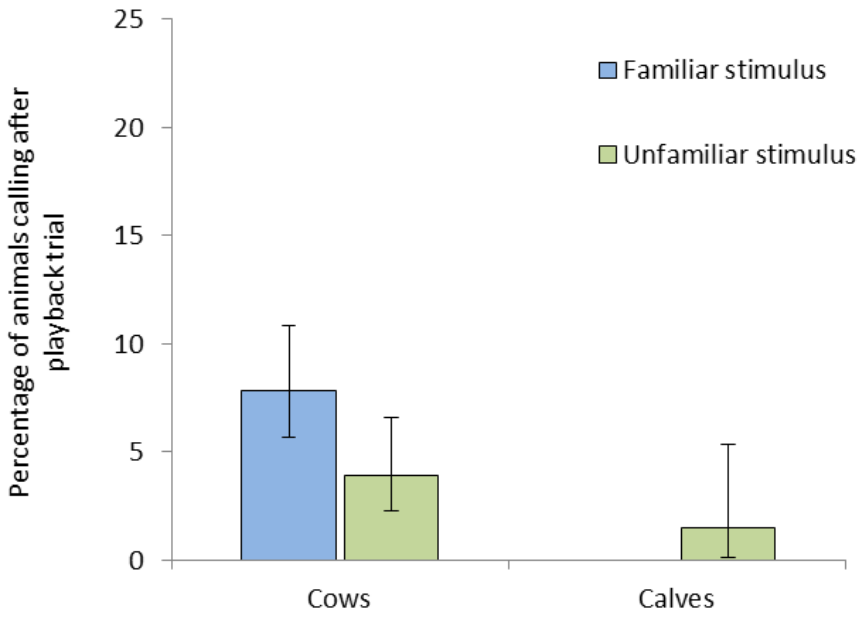

Figure 4.10. Percentage of cows and calves calling after familiar and unfamiliar playback trials.

4.5 Discussion

I investigated the individual behavioural responses of cattle when exposed to different visual and acoustic stimuli in unconstrained semi-natural conditions. The results of the novel object test provide evidence of consistent behavioural differences between cows and calves. Calves were generally more responsive when presented with novel objects, displaying both more apparently bold behaviours (e.g. sniffing or licking the object) and more apparently shy behaviours (e.g. running away from the object). Contrary to the prevailing views in the literature and to my prediction, however, no additional significant effect of individually was found. By contrast, the results of the heterospecific playback experiment revealed only limited differences in the behavioural responses of cows and calves, but the existence of marked individual behavioural consistency on the propensity of cattle to look at and approach the speaker.

Overall, these results provide tentative support for the findings of other studies of cattle (Boissy & Bouissou 1995), and more generally for domestic ungulates (e.g. goats, Lyons et al. 1988; sheep, Romeyer and Bouissou, 1992), which suggest that individual differences in temperament might have an important role in how animals react to novel situations and the threat of predation (Jones & Godin 2010), and in general how they behave in a variety of different social contexts (Réale et al. 2000). Characterizing these differences is therefore fundamental to a

proper understanding of the adaptive significance of cattle behaviour, and has considerable potential value to those seeking to improve productivity (Muller & von Keyserlingk 2006) and welfare.

4.5.1 Experiment 1

On the basis of the PC's generated with the behavioural responses to three different objects, I was able to classify cows and calves as relatively bold or relatively shy. Although calves showed the greatest tendency to explore the new objects, they also showed the greatest tendency to avoid them (Figure 4.4). This could possibly be explained because novel situations can simultaneously present naïve animals with the potential for both benefits and costs. The objects presented to animals in this experiment may have been perceived as potentially rewarding or potentially risky by different individuals. As a result, young animals, lacking experience of such novel situations, may have tended to be both relatively more "curious" about potentially rewarding objects, and more fearful about potential risks in their environment, than older, more experienced adults. In order to measure individual consistency in a particular behaviour, it is necessary to measure that behaviour repeatedly. However, most previous studies of cattle temperament have in fact either not replicated measurements of responses to stimuli at all (Boisssy & Bouissou 1995; Herskin et al. 2004; Gibbons et al. 2009), or have only assessed behaviour twice (Jones & Godin 2010; Kurvers

et al. 2010). One set of experiments which did have proper replication (Van Reenen et al. 2004 & 2005) demonstrated that calf behaviours were consistent across development (at 3, 16 and 29 weeks old), revealing the existence of stable characteristics mediating reactivity to potentially alarming situations (human presence, novel objects and open field tests; Van Reenen et al. 2004) and stress (novel environments and novel objects; Van Reenen et al. 2005). The failure to detect significant individuality in the novel object experiment in the present study is likely to have arisen because of subtle differences in experimental design, or because variance in temperament among individuals in cattle is not universal across populations and contexts.

Unlike in some previous studies of behavioural responses to novel objects, where the same object was presented to individuals repeatedly (Van Reenen et al. 2004 & 2005; Kligour et al. 2006; Jones & Godin 2010; Kurvers et al. 2010), replicates in this novel object experiment were performed with different objects. Each trial therefore provided a slightly different context in which behaviour was measured. It seems unlikely, however, that this difference in experimental design was in any sense responsible for the absence of detectable individuality in my novel object experiment, since the random effect of object type on responses was not significant.

4.5.2 Experiment 2

I evaluated both the behavioural response of the whole herd (not accounting for individuality), and known individuals, to different heterospecific recordings that were played back in 27 different trials. First, with a PCA, I was able to classify the behavioural responses of the whole herds as either "bold" or "shy". PC1 included all bold behaviours (looking, calling and approaching) and PC2 included the shy behaviour (moving away). However, the subsequent statistical analysis showed no significant effects of either the familiarity of the stimulus being played, or of its identity, on either PC. Thus, there was no evidence at the level of the herd that cattle were sensitive to the differences between sounds which they frequently encounter in their natural environment, and unfamiliar sounds which could represent a significant threat. However, this analysis ignored the possibility that responses of cattle to the playbacks might be influenced by individual temperament.

On the other hand, a subsequent individual-level analysis of three different behavioural responses (looking, approaching and calling) to playbacks of heterospecific vocalizations revealed the existence of individual behavioural consistency in cattle. Different individuals had different tendencies to look at and approach the loudspeaker after playbacks, irrespective of the familiarity or identity of the playback sequence (Figure 4.5 and Figure 4.7, respectively). Owing

the nature of the data, it was necessary to employ a relatively more crude binomial classification of responses of individuals in this experiment than when considering the response at the level of the herd, or indeed than in the novel object experiment. Nevertheless, the results are broadly consistent with the hypothesis that individuals vary in the extent to which they are bolder or shy in response to potentially rewarding or threatening stimuli, and hence provide some tentative support for the existence of temperament in cattle in semi-natural situations.

The individual level analysis of responses to heterospecific playbacks also revealed a strong random effect of individual trial on all cattle responses (looking, approaching and calling). In other words, when one animal looked, approached or called, others were more likely to do the same, irrespective of the nature of the stimulus, or their temperament. This could suggests that the behavioural response of individuals to stimuli is strongly influenced by the responses of other members of the herd. Socially-mediated behaviour of this sort, in particular in response to potential threats, has previously been reported in other ungulates, where animals tend to gather as a defence mechanism (Sibbald et al. 2009). Indeed, group living may have evolved partially as a response to predation pressure (Mendl and Held 2001). In prey species, which include most large herbivores, fear of predators is considered to be a major factor in the formation

and maintenance of groups (Sibbald et al. 2009), and in this context the collective behaviours exhibited by cattle in my playback experiment are not surprising.

Interestingly, contrary to expectations, there was no statistical evidence that the responses of cattle, at either at the level of the herd or the individual, were affected by the familiarity or the identity of the heterospecific individual whose call was played back to them. Thus, for example, while cattle were most likely to look and approach the speaker when they heard the playback of one of the farmer's voices (Catherine) and the wolves howling (See Figures 4.6 and Figure 4.8), there was no evidence that this reflected anything other than the strong random effect of individual trial on the response. This contradicts anecdotal evidence (MPT personal observations) that the cattle were apparently able to associate the farmer's voice with a potential food reward, and the wolf howls with an unknown threat. The playbacks of the wolves howling in particular seemed to elicit uniquely strong reactions from the cattle, with the whole herd rushing towards the speaker, and many individuals displaying apparently aggressive head-shaking behaviour. This could be because wolves were identified as a more potent threat than dogs or humans. Indeed, there is evidence that cattle are able to differentiate between two predators, the wolf and the mountain lion (*Puma concolor*), using visual and olfactory stimuli (Kluever et al. 2009), and it has been reported elsewhere that cattle modify their behaviour in relation to the presence of wolves (Laporte et al. 2010). Unfortunately, it was not

possible to score consistently and reliably from the videos of trials either head-shaking, or the difference between a steady approach and a rushed approach to the loudspeaker. In addition, it was only possible to perform three replicates with each stimulus, and only one wolf recording was used. Thus, it remains entirely possible that the apparent differences in responses observed in the field were simply a reflection of the considerable variation in behaviour of the herd from trial to trial.

4.6 Conclusions

In domestic animals, the opportunity to express normal social behaviours is usually limited by captivity and husbandry systems, which could lead to poor welfare (Miranda-de la Lama & Mattiello 2010). In order establish the ideal (basal) individual behavioural responses in cattle under intensive husbandry conditions, it is necessary to characterise “normal” behaviour in natural or semi-natural free-range conditions. Similarly, in order to understand the role that temperament plays in determining individual behaviour in domestic animals, more research under the most natural possible conditions should be performed. This was a prime motivation for the present study. Overall, the findings contribute to a growing understanding of animal temperament in cattle and, more generally, of the role of individuality in animal behaviour. Further such work is going to be critical in the near future if we are to make progress in understanding animal welfare and applying knowledge of animal behaviour in order to maximise productivity.

4.7 References

Albright, J. L., & Arave, C. W. 1997. The behaviour of cattle. CAB international, Wallingfrod, UK, 306 pp.

Anonymous 2010. WolfCountry net: http://www.wolfcountry.net/WolfSounds.html

Atwell, J.W., Cardoso, G.C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K.W., & Ketterson, E.D. 2012. Boldness behaviour and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. Behavioural Ecology 25, 960-969.

Bell, A. M. 2007. Future directions in behavioural syndromes research. Proceedings of the Royal Society B 274, 755-761.

Bell, A.A. 2009. The repeatability of behaviour: a meta-analysis. Animal Behaviour 77, 771-783.

Biro, P.A. & Stamps, J.A. 2008. Are animal personality traits linked to life-history productivity? Trends in Ecology and Evolution 23, 361–368.

Boersma P, Weenink D. 2009. Praat: doing phonetics by computer. http:www.praat.org/

Bouissou, M. F. 1972. Influence of body weight and presence of horns on social rank in domestic cattle. Animal Behaviour 20, 474-477.

Bouissou, M. F., Boissy, A., Le Neindre, P., & Veissier, I. 2001. The social behaviour in cattle. In: Keeling, L. J., & Gonyou, H. W. Social behaviour in farm animals. CABI Publishing. Oxon, UK. pp. 113-145.

Boissy, A., & Bouissou, M.F. 1995. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Applied Animal Behaviour Science 46, 17-31.

Burrow, H.M. 1997. Measurements of temperament and their relationship with performance traits of beef cattle. Animal Breeding Abstracts 65, 477-495.

Burrow, H.M., & Dillon, R.D. 1997. Relationships between temperament and growth in a feedlot and commercial carcass traits of *Bos indicus* crossbreeds. Australian Journal of Experimental Agriculture 37, 407-411.

Broom, D.M. 1988. The scientific assessment of animal welfare. Applied Animal Behaviour Science 20, 5-19.

Carere, C., Drent P.J. Privitera, L. Kookhaas, J.M., & Groothuis GGT. 2005. Personalities in great tits, *Parus major*: stability and consistency. Animal Behaviour 70, 775-805.

Carter, A.J., Goldizen, A.W., Tromp, S.A. 2010. Agamas exhibit behavioural syndromes: bolder males bask and feed more but may suffer higher predation. BehavioralEcology 21, 655-661

Carter, A.J. Heinsohn, R. Goldizen, A.W., & Biro, P.A. 2012. Boldness, trappability and sampling bias in wild lizards. Animal Behaviour 83, 1081-1058.

Carter, A.J., Marshall, H.H., Heinsohn, R., & Cowlishaw, G. 2012. Evaluating animal personalities: do observer assessments and experimental test measure the same thing? Behavioural & Ecology Sociobiology 53, 153-160.

Clark, A.B., & Ehlinger, T.J. 1987. Pattern and adaptation in individual behavioural differences. In: Perspectives in Ethology. Bateson, P.P.G., & Klopfer, P.H. (Eds.) Plenum Press, New York, pp-1-47.

Coulon, M., Deputte B.L., Heyman, Y., Richard, C., & Delatouch, L. 2007. Visual discrimination by heifers (*Bos taurus*) of their own species. Journal of Comparative psychology 121, 198-204.

Coulon, M., Deputte B.L., Heyman, Y., & Baudoin C. 2009. Individual recognition in domestic cattle (*Bos taurus*): Evidence from 2D- Images of heads from different breeds. PLoS One 4, 1-8.

Dingemanse, N.J., Kazem, A.J.N., Reale, D., & Wright, J. 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology and Evolution 25, 81-89.

Drews, C., 1993. The concept and definition of dominance in animal behaviour. Behaviour 125, 283–313.

Fordyce, G. E., & Goddard, M.E. 1984. Maternal influence on the temperament of *Bos indicus*-cross cows Proc. Australian Society of Animal Production 15, 345-348.

Gibbons, J., Lawrence, A., & Haskell, M. 2009. Responsiveness of dairy cows to human approach and novel stimuli. Applied Animal Behaviour Science 116, 163-173.

Hagen K., Broom, D.M. 2003. Cattle discriminate between individual familiar herd members in a learning experiment . Applied Animal Behaviour Science 82, 13-28.

Harcourt, J.L., Sweetman, G., Johnstone, R.A., & Manica A. 2009. Personality counts: the effect of boldness on shoal choice in three-spined sticklebacks. Animal Behaviour 77, 1501-1505.

Hemsworth, P.H., Coleman, G.J., Barnett, J.L., & Sorg, S. 2000. Relationship between human-animal interaction and productivity of commercial dairy cows. Journal of Animal Science 78, 2821-2831.

Herskin, M. S., Kristensen, A.M., Munksgaard, L. 2004. Behavioural responses of dairy cattle toward novel stimuli presented in the home environment. Applied Animal Behaviour Science 89, 27-40.

IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. IBM Corp. Armonk, NY, USA.

Jones, K.A., & Godin, J.G.J. 2010. Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. Proceedings of the Royal Society B. 277, 625-632.

Kilgour, R.J., Melville, G.J., & Greenwood, P.L. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans and novelty. Applied Animal Behaviour Science 99, 21-40.

Kluever, B.M., Howery, L.D., Breck, S.W., & Bergman, D.L. 2009. Predator and heterospecific stimuli alter behavior in cattle. Behavioral Processes 81, 85-91.

Kurvers, R.H.J.M., van Oers, K., Nolet, B.A., Jonker, R.M., van Wieren, E., Prins, H.H.T., & Ydenberg, R.C. 2010. Personality predicts the use of social information. Ecology Letters 13, 829-837.

Laporte, I., Muhly, T.B., Pitt, J.A., Alexander, M., & Musiani, M. 2010. Effects of wolves on elk and cattle behaviours: implications for livestock production and wolf conservation. PLos One. 5, e11954.

Le Neindre, P., Trillat, G., Sapa, J., Menissier, F., Bonnet, J.N. and Chupin, J.M. 1995. Individual differences in docility in limousin cattle. Journal of Animal Science 73**,** 2249-2253.

Lyons, D.M., Price, E.O., & Moberg, G.P. 1988. Individual differences in temperament of domestic dairy goats: constancy and change. Animal Behaviour 36, 1323-1333.

Manteca, X., Deag, J.M. 1993. Individual differences in temperament of domestic animals: a review of methodology. Animal Welfare 2, 247-268.

Mathot, K.J. Wright, B., Kempenaers, J., & Dingemanse, N.J. 2012. Adaptative strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. Oikos 121, 1009-1020.

Mendl, M., & Held, S. 2001. Living in groups: an evolutionary perspective. In: Keeling, L.J., Gonyou, H.W. (Eds.) Social behaviour in farm animals. Cabi Publishing. Wallingford, pp. 7-39.

Miranda-de la Lama, G.C., & Mattiello, S. 2010. The importance of social behaviour for goat welfare in livestock farming. Small Ruminant Research 90, 1-10.

Müller, R. & von Keyserlingk, M.A.G. 2006. Consistency on flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. Applied Animal Behaviour Science 99, 193-2004.

Müller, R & Schroeder, L. 2005a. Individual consistency of dairy cows' activity in their home pen. Journal of Dairy Science 88, 171-175.

Müller, R & Schroeder, L. 2005b. Behavioural consistency during social separation and personality in dairy cows. Behaviour 142, 1289-1306.

Patherick, J.C., Holroyd, R.G., Doogan, V.J., & Venus, B.K. 2002. Productivity, carcass and meat quality of lot -fed *Bos indicus* cross steers grouped according to temperament. Australian Journal of Experimental Agriculture 42, 389-398.

R Development Core Team 2009. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org.

Rèale, D. Gallant, B.Y., Leblanc, M., & Festa-Bianchet, M. 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. Animal Behaviour 60, 589-597.

Rèale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews 82, 291-318.

Romeyer, A., & Bouissou, M.F., 1992. Assessment of fear reactions in domestic sheep and influence of breed and rearing conditions. Applied Animal Behavioural Science 34, 93-l 19.

Sibbald, A. M., Erhard, H.W., McLeod, J.E., & Hooper, R.J. 2009. Individual personality and the spatial distribution of groups of grazing animals: An example with sheep. Behavioural Processes 82, 319-326.

Sih, A. 2004. Behavioural syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution 19, 372-378.

Sih, A., & Watters, J.V. 2005. The mix matters: behavioural types and group dynamics in water striders. Behaviour 142, 1417-1431.

Sih, A., Kats, L.B., & Maurer, E.F. 2003. Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. Anim. Behaviour 65, 29–44.

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. 2012. Ecological implications of behavioural syndromes. Ecology Letters 15, 278-289.

Smith, B.R. & Blumstein, D.T. 2008. Fitness consequences of personality: a meta-analysis. Behavioural Ecology 19, 448–455.

Stamps, J.A. 2007. Growth-mortality tradeoffs and "personality traits" in animals. Ecology Letters 10, 355-363.

Stamps, J. A., & Groothuis, T.G.G. 2010. The development of animal personality: relevance, concepts and perspectives. Biological Reviews 85, 301-325.

Van Reenen, C.G., O'Connell, N.E., Van der Werf, J.T.N., Korte, S.M., Hopster, H., Jones, R.B., & Blokhuis, H.J. 2005. Responses of calves to acute stress: Individual consistency and relations between behavioural and physiological measures. Physiology & Behaviour85, 557-570.

Van Reenen, C.G., Engel, B., Ruis-Heutinck, L.F.M., Van der Werf, J.T.N., Buist, W.G., & Blokhuis, H.J. 2004. Behavioural reactivity of heifer calves in

potentially alarming test situations: a multivariate and correlational analysis. Applied Animal Behaviour Science 85, 11-30.

Welp, T., Rushen, J., Kramer, D.L., Festa-Bianchet, M., de Pasitlle, A.M.B. 2004. Vigilance as a measure of fear in dairy cattle. Applied Animal Behaviour Science 87, 1-13.

Wilson, D.S. 1998. Adaptative individual differences within single populations. Philosophical Transactions of the Royal Society B 353, 199-205.

Wilson, A.D.M, & Godin, J.G.J. 2009. Boldness and behavioral syndromes in the bluegill sunfish, Lepomis macrochirus Behavioral Ecology 20, 231-237.

Wilson, D.S., Coleman, K., Clark, A.B., & Biederman, L. 1993. Shy bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*)—an ecological study of a psychological trait. Journal of Comparative Psychology 107:250–260.

Wilson, D.S, Clark, A.B., Coleman, K.,& Dearstyne, T. 1994. Shyness and boldness in humans and other animals. Trends in Ecology and Evolution 9,442–446.

Wolf, M., & Weissing, F.J. 2012. Animal personalities: consequences for ecology and evolution. Trends in Ecology and Evolution 27, 452-461.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A.A., & Smith, G. M. 2009. Mixed effects and extensions in ecology with R. Springer, NY, USA. 574 pp.

CHAPTER FIVE

Correlations among behaviours in cattle: vocalizations, mother-offspring recognition and temperament

5. Correlations among behaviours in cattle: vocalizations, mother-offspring recognition and temperament

5.1 Introduction

It is known that temperament or personality traits play a significant role in behavioural decisions in animals and are linked to foraging efficiency, mating behaviour and life history traits such as dispersal and reproductive success (Reale et al. 2007; see Chapter 4). It has been proposed that nonhuman animals can be ranked on a shy-bold continuum (Sih 2004), exhibiting relatively consistent responses across different contexts and environments. Thus, an individual responding as bold in social interactions is expected to behave in a bold manner in threatening situations, during foraging, and during mating (Harvey & Freeberg 2007). Furthermore, the position of an individual on the bold-shy continuum has been shown to correlate with other morphological and physiological traits. For example, the boldness of antipredator and exploratory responses is significantly influenced by body size in the sand fiddler crab *Uca pugilator* (Decker & Griffen 2012). Similarly, it has been shown that in greylag geese (*Anser anser*) aggressiveness positively correlates with stress-induced corticosterone levels, heart rate, body size, and with dominance rank (Kralj-Fiser & Weiβ 2010). This evidence suggests that boldness is indicative of more general and far-reaching biological individuality within populations.

There is growing evidence, at least from studies of birds, that personality traits correlate with the characteristics of an individual's vocalizations. Male singing behaviour in songbirds varies systematically with personality traits such as exploration and risk taking. Naguib et al. (2010) found that male great tits (*Parus major*) were not only consistent in the overall number of songs produced and the time of the day during which they sang, but also that males that sang more songs also tended to have higher explorations scores in a novel environment. Additionally, it has been shown that in black-capped chickadees (*Poecile atricapillus*) an individual's vocal output, including calls and songs, is consistent across contexts and over time, and correlates with aspects of personality (Guillette & Sturdy 2011). Exploratory behaviour of individual birds in a novel environment task was positively associated with the propensity to vocalize during motor behaviour in two different contexts: a stressful condition where chickadee mobbing calls were played to individual birds, and a control condition with no playbacks.

Temperament has an important effect on cattle responses to human handling (Burrow 1997), and as a result a number of diagnostic tests have been developed to help farmers characterise the temperament of their animals. However, to date there is no real consensus about a definitive set of criteria for the assessment of temperament. Reactions to stimuli indicating potential threats (e.g. those associated with predators) in farm animals can be used to predict an individual's

ability to adapt to the constraints of husbandry, and thus to improve the efficiency of production and possibly the welfare (Boissy & Bouissou 1995). Responses to other stimuli might be equally informative. For example, behavioural responses to novel objects are reported to be good indicators of the bold-shy behavioural syndrome, and are known to correlate with a suite of other behavioural, morphological and physiological traits (e.g. in lizards, *Agama planiceps*, Carter et al. 2012; in sticklebacks, *Gasterosteus aculeatus,* Harcourt et al. 2009; see also review by Rèale et al. 2007). However, very few attempts have been made to characterise temperament in farm animals by testing the consistency of, and correlations among, individual responses to threatening stimuli (Welp et al. 2004; Kluever et al. 2009; Laporte et al. 2010), novel objects (Herskin et al. 2004; Kilgour et al. 2006; Gibbons et al. 2009) and other traits, such as vocalisations (Boissy & Boussiou 1995; Forkman et al. 2007; but see in pigs Van Kooij et al. 2002). The extent to which existing studies are informative about individuality in cattle behaviour is also limited by the conditions in which most of the experiments have been conducted, where animals were usually confined and socially isolated (and hence probably under considerable stress).

The link between variations in vocal parameters and emotion-related (arousal) physiological changes in the vocal apparatus has rarely been investigated (see review Briefer 2012). Nevertheless, it is known that emotions induce changes in the somatic and autonomic nervous system, which in turn cause tension and

action of muscles used for voice production, as well as changes in respiration rates and salivation, and hence changes in the acoustic properties of vocalisations (Scherer 2003; Zei Pollermann & Archinard 2002). Furthermore, there is evidence that some kinds of vocalisation can encode subtle information about the state of the individual. For example, numerous studies have concluded that alarm calls can include information about the urgency of the threat and/or the type of predator (Macedonia & Evans 1993; Manser 2001; Leavesley & Magrath 2005).

Although contact calls have a different function from alarm calls, they too have the potential to encode information about not only about the identity of the caller (see Chapter 2), but also its emotional state. Specifically, because of the context in which they are generated (after varying periods of separation between mother and offspring in herds of varying sizes and habitats of varying complexity), they may contain information about an individual's state of arousal due to the urgency to localize either their mother or their offspring. Similarly, the propensity of an animal to respond to its parent or offspring is also likely to reflect this state of arousal. In turn, the behavioural response to separation, and hence the associated state of arousal, seems likely to reflect personality/temperament, and thus it may be the case that other indicators of personality, such as responses to encounters with novel objects, correlate with attributes of contact calls and the behavioural responses they elicit.

Although we know about the acoustic characteristics of mother-offspring calls and their ability to individually distinguish their own mother-offspring in several mammal species (sheep; Searby & Jouventin 2003; fallow deer; Torriani et al. 2006; goat; Briefer & McElligott 2011), no research has been done linking the acoustic characteristics mother-offspring contact calls, the behavioural responses to those calls, and other aspects of temperament.

5.2 Objectives

There is currently very little literature available linking animal personality or temperament with acoustic characteristics. In this chapter, for the first time to my knowledge, I looked for associations between behavioural responses of cattle to visual and acoustic stimuli, the acoustic characteristics of their vocalizations (specifically, contact calls), and their ability to recognize and respond to their own mother or offspring on the basis of acoustic cues only. This was achieved by combining and analysing the results obtained from the previous chapters of this thesis to determine if there were associations indicative of consistent individual variation across widely differing behavioural contexts.

5.3 Methodology

In order to determine if there are any possible links between cattle temperament and their vocalizations, I carried out a series of correlations among the variables obtained in each of the experiments presented in earlier chapters. The data used were: acoustic characteristics from contact calls in cows (both low frequency, LFCs, and high frequency, HFCs) and calves (Chapter 2); behavioural responses obtained from mother-offspring experiment evidencing bidirectional recognition (Chapter 3); behavioural responses to novel objects under unconstrained conditions (Chapter 4, Section 4.3.2); and finally behavioural responses given by cattle to auditory stimuli presented in the field (Chapter 4, Section 4.3.5).

5.3.1 Statistical analysis

In order to determine if a relationship existed between the acoustic characteristics (Fundamental frequency, F0, and minimum spacing of formats Df(min)) of cow contact calls (LFCs and HFCs), and the ability of cattle to identify their own mother/offspring, I carried out a series of T-tests of the differences between responders to playbacks in the mother/offspring recognition experiment and non-responders analysis (IBM SPSS version 20, 2011). Responses to playbacks of Own and Other individuals were considered separately (see Section 3.3.4 for definitions). Only the behavioural response which provided the strongest statistical contrast between responders and non-responders was

considered; for responses to Own individuals, this was directing the body towards the loudspeaker or standing up, whilst for responses to Other individuals this was ear movements or looking towards the loudspeaker. For other behavioural responses, no meaningful contrast was possible because most or all animals responded in the same way to the playbacks. Calves were not considered in this analysis, because all the necessary data were available for only two animals.

In order to determine if there was an association between the acoustic characteristics of cattle vocalisations and the behavioural responses towards visual (novel object) and auditory (heterospecific playbacks) stimuli, I carried out Pearson's correlations using the principal components (PCs) describing individual behavioural responses to the novel object experiment (Chapter 4, Table 4.3) and auditory stimuli experiment (see below), and F0 (mean fundamental frequency) and Df(min) (minimum formant spacing) from LFCs, HFCs and calf calls. To perform this analysis, the PC scores describing behavioural responses in the three trials to which each animal in the novel object experiment was subjected were averaged. For the responses in the auditory stimuli experiment, a new Principal Components Analysis (PCA) was conducted on data generated by pooling responses of individuals across all trials in which they were visible on camera analysis (IBM SPSS version 20, 2011). This pooling of data was logical, given that the fixed effect of stimulus familiarity and the random effect of stimulus identity were not significant in the analysis in Chapter 4 (Section 4.4.2). Calculating the

proportion of trials (up to a maximum of 27) in which each animal expressed each of four behaviours (looking at and approaching the loudspeaker, calling, and moving away) created a dataset with four correlated variables which could then be subject to PCA. The PCA created two new PCs describing the general response of individuals to the auditory stimuli, the scores from which were then used in the correlations outlined above.

In order to determine if there was an association between the ability of cows and calves to recognize and respond to their own mother/offspring, and the behavioural responses towards visual (novel object) and auditory (heterospecific playbacks) stimuli, I first carried out a Spearman's rank correlation analysis (IBM SPSS version 20, 2011) using the PCs describing the responses of individuals to novel objects and auditory stimuli (see above), and the strength of the response to mother/offspring for Own and Other individuals, measured on a scale of 1 to 4 (see Section 3.3.5 in Chapter 3). In a second approach, T-tests were carried out in order to determine if there was an association between the ability of cows and calves to recognize their own mother/offspring, and the behavioural responses towards the visual (novel object) and auditory (heterospecific playbacks) stimuli. As above, responders and non-responders were compared, with responses to Own and Other individuals being considered separately.

Finally, a Pearson's correlation analysis (IBM SPSS version 20, 2011) between the PCs describing individual responses in the novel object experiment (Chapter 4, Table 4.3) and the auditory stimuli experiment (Chapter 4, Table 4.4) was also performed in order to find out if individual behaviour in these two rather different contexts was correlated.

Throughout these analyses, sample sizes are strongly constrained by the availability of all the necessary data for individuals. Sample sizes vary among analyses because different combinations of individuals were used in different experiments.

5.4 Results

There were no significant differences between responders and non-responders animals in the mother/offspring recognition experiment in the acoustic parameters (F0 and Df(min)) of cow HFCs and LFCs, irrespective of whether responses of Own (Table 5.1) or Other (Table 5.2) animals were considered.

Table 5.1. T-tests of differences in mean F0 and Df(min) between responders and non-responders in mother/offspring recognition experiments assessed for **Own** animals (Chapter 3, Section 3.3.4). Only the behavioural response (directing the body towards the loudspeaker or standing up) which provided the most powerful statistical comparison was used. Sample sizes were n = 6 cows (LFCs) and n = 10 cows (HFCs); mean acoustic characteristics were calculated from 7 – 10 calls per animal.

	T	**df**	**P (2-tailed)**
LFCs F0	-0.457	4	0.671
LFCc Df (min)	0.918	4	0.410
HFCs F0	0.274	8	0.791
HFCs Df (min)	1.032	8	0.332

Table 5.2. T-tests of differences in mean F0 and Df(min) between responders and non-responders in mother/offspring recognition experiments assessed for **Other** animals (Chapter 3, Section 3.3.4). Only the behavioural response (ear movements and looking towards the loudspeaker) which provided the most powerful statistical comparison was used. Sample sizes were n = 6 cows (LFCs) and n = 10 cows (HFCs); mean acoustic characteristics are calculated from 7 – 10 calls per animal.

	T	**df**	**P (2-tailed)**
LFCs F0	2.033	4	0.112
LFCc Df (min)	0.418	4	0.697
HFCs F0	0.917	8	0.386
HFCs Df (min)	0.916	8	0.386

There was limited evidence that individual responses to novel objects were associated with the characteristics of animal´s vocalizations (Table 5.3). Correlations performed between the acoustic components F0 (mean) and Df (min) describing the LFCs, HFCs and calf calls, and the PCs obtained from the behavioural responses to novel objects showed that there was a significant positive association between Df(min) of cow LFCs and the second principal component (PC2) describing responses to novel objects (Figure 5.1). Since PC2 was positively correlated with calling and sniffing behaviours, it could be considered to be indicative of bold behaviours when presented with a novel

object and hence this result tentatively suggests that bolder cows had vocalisations with higher Df (min).

Table 5.3. Correlations between the acoustic parameters (F0 (mean) and Df (min)) of LFCs, HFCs and calf calls (means of 7 – 10 calls per individual cow, and 9 – 10 calls per calf), and the PC scores (means from 3 trials per individual) obtained from the behavioural responses to novel objects in the field (Chapter 4).

			Adult cow calls			Calf calls	
		LFCs F0 (mean)	LFCs Df (min)	HFCs F0 (mean)	HFCs Df (min)	Calves F0 (mean)	Calves Df (min)
Principal components describing responses to novel objects	PC1	Pearson's Correlation	0.442	-0.485	-0.024	-0.034	-0.602	-0.453
		P (2-tailed)	0.321	0.271	0.945	0.921	0.206	0.367
		N	7	7	11	11	6	6
	PC2	Pearson's Correlation	-0.045	0.865*	-0.010	-0.301	0.144	0.435
		P (2-tailed)	0.924	**0.012**	0.978	0.369	0.786	0.389
		N	7	7	11	11	6	6
	PC3	Pearson's Correlation	-0.250	-0.571	-0.095	-0.311	0.358	0.276
		P (2-tailed)	0.588	0.181	0.780	0.351	0.486	0.596
		N	7	7	11	11	6	6

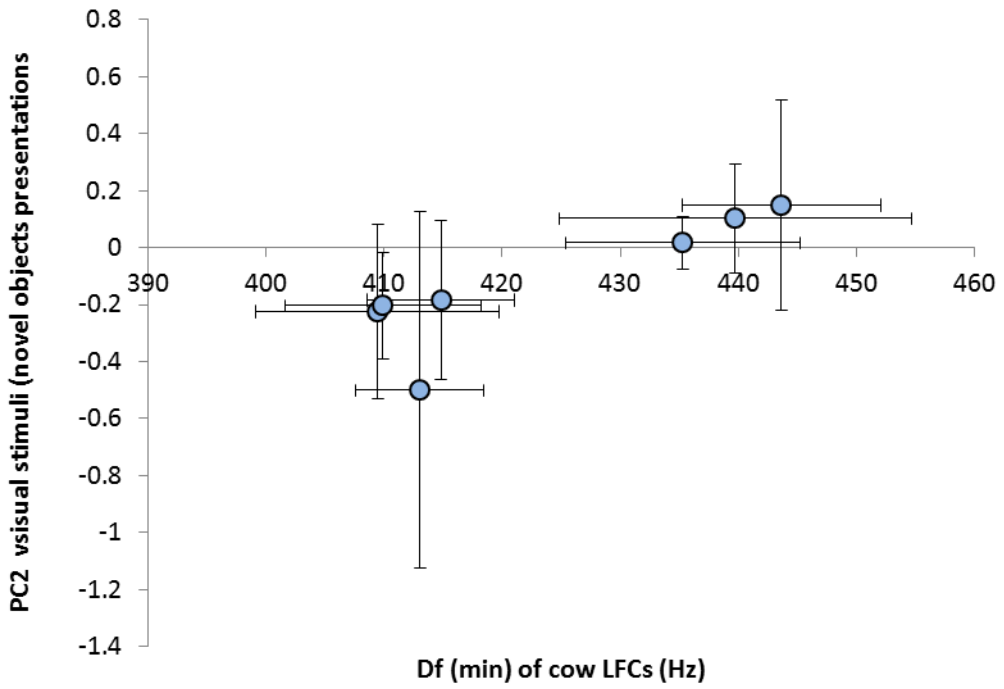

Figure 5.1. Correlation between mean Df (min) (+/- SEM) of cow LFCs (n = 7 – 10 calls per animal) and mean scores of the second principal component (PC2) (means from 3 trials per individual) (+/- SEM) describing behavioural responses to novel objects presented in the field (Chapter 4, Section 4.4.1).

The additional PCA of responses to auditory stimuli from Chapter 4 generated two new PCs, which together explained 62.7 % of the variance in the original data-set (Table 5.4). The first component correlated positively with the proportion of trials in which individuals called or approached the loudspeaker, while the second component correlated negatively with the proportion of trials in which animals moved away from the loudspeaker. When I tested for correlations between these new PCs describing behavioural responses to auditory stimuli, and the acoustic characteristics of cows (HFCs and LFCs) and calf vocalizations, I found a positive correlation between mean Df(min) of cow LFCs and PC2 (Table 5.5;

Figure 5.2), which was similar to the correlation seen above (Figure 5.1) between Df(min) and PC2 from the novel object experiment. This again suggests that bold animals, which had high scores on PC2 (i.e. were less likely to move away from the loudspeaker), had LFCs with higher Df (min) (Figure 5.2).

Table 5.4. Results of Principal Component Analysis (PCA) of behavioural responses to heterospecific audio playbacks (pooled across up to 27 playback trials per individual). Bold types indicates loadings > 0.5.

	Component	
	PC1	PC2
Proportion looking	**0.727**	0.139
Proportion calling	**0.703**	0.481
Proportion approaching	-0.562	0.378
Proportion moving away	0.271	**-0.838**

Table 5.5 Correlations between the acoustic parameters (F0 (mean) and Df (min)) of LFCs, HFCs and calf calls (means of 7 – 10 calls per animal) and PC scores (calculated from pooled behavioural responses from up to 27 playback trials per individual) describing the behavioural responses to auditory stimuli (heterospecific playbacks) in the field (Chapter 4, Section 4.4.2).

			Adult cow calls			**Calf calls**	
		LFCs F0 (mean)	**LFCs Df (min)**	**HFCs F0 (mean)**	**HFCs Df (min)**	**Calves F0 (mean)**	**Calves Df (min)**
Responses to auditory stimuli	Pearson's Correlation	0.548	-0.030	-0.296	0.227	-0.725**	0.067
PC1	P (2-tailed)	0.160	0.943	0.326	0.455	**0.005**	0.829
	N	8	8	13	13	13	13
	Pearson's Correlation	-0.456	0.743*	-0.402	-0.041	0.369	0.169
PC2	P (2-tailed)	0.256	**0.035**	0.173	0.894	0.215	0.582
	N	8	8	13	13	13	13

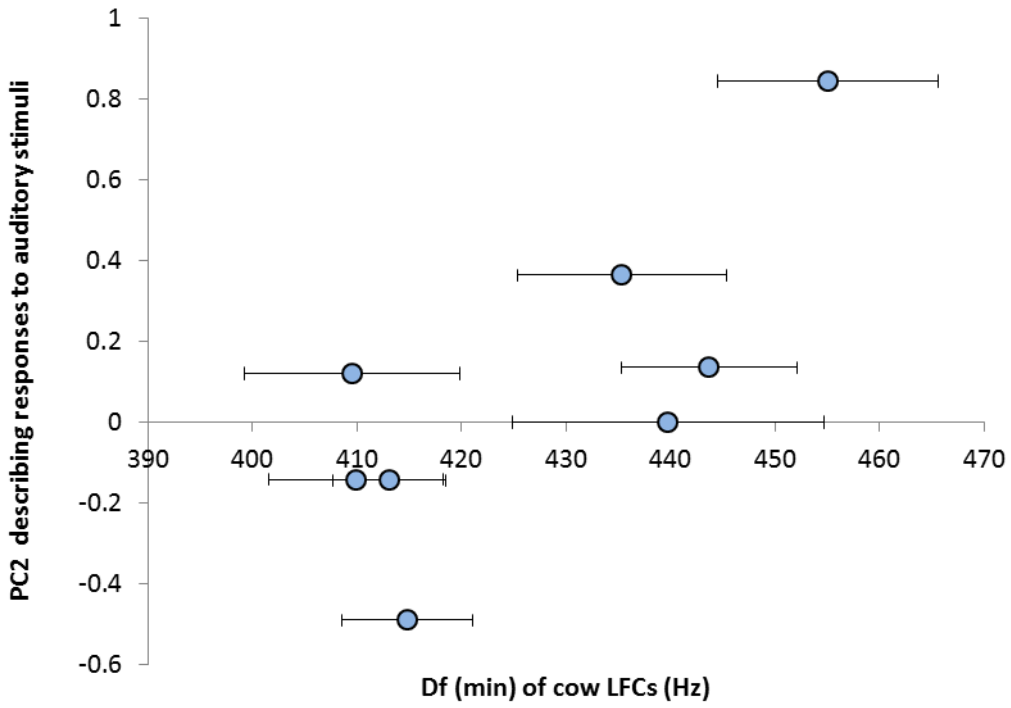

Figure 5.2. Correlation between mean Df (min) (+/- SEM) of cow LFCs (n = 7 – 9 calls per animal) and the score for the second principal component (PC2) (calculated from responses pooled across up to 27 playback trials per individual) describing behavioural responses to auditory stimuli (paybacks of heterospecific vocalisations; see Chapter 4, Section 4.4.2).

A negative correlation was also found between the mean F0 of calf calls and PC1 describing the responses to auditory stimuli, which in this case grouped the boldest behaviours (Table 5.5; Figure 5.3), suggesting that bolder calves had lower F0.

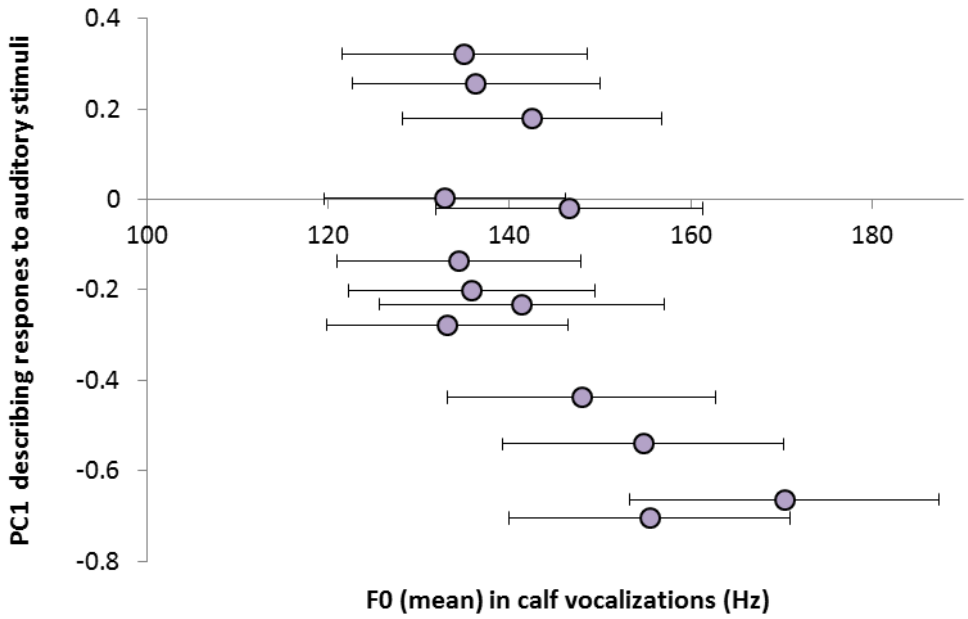

Figure 5.3. Correlation between mean F0 (mean) (+/- SEM) of calf vocalizations (n = 9 – 10 calls per animal) and scores for the second principal component (PC2) (calculated from responses pooled across up to 27 playback trials per individual) describing behavioural responses to auditory stimuli (paybacks of heterospecific vocalisations; see Chapter 4, Section 4.4.2).

There were no statistically significant correlations between PC scores describing the behavioural responses of individuals to novel object presentations (Chapter 4, Section 4.4.1) and the strength of behavioural responses to playbacks of conspecific calls from Own and Other individuals (Chapter 3; Table 5.6). The same was true for correlations between the PC scores describing the behavioural responses of individuals to the auditory stimuli (heterospecific playbacks; Chapter 4, Section 4.4.2) and the strength of behavioural responses to playbacks of conspecific calls from Own and Other individuals (Chapter 3; Table 5.7). Thus,

there was no evidence that the ability of cattle to individually identify their own mother/offspring is related to the way they respond to visual and auditory stimuli presented in the field.

Table 5.6 Spearman´s rank correlations of principal component (PC) scores (means from 3 trials per individual) describing the responses of cattle to novel object presentations, and the strength of the response to mother/offspring calls from Own and Other individuals.

			Own animal scores	**Other animal scores**
Responses to novel objects	**PC1**	Correlation Coefficient	0.164	-0.021
		P (2-tailed)	0.575	0.947
		N	14	13
	PC2	Correlation Coefficient	-0.308	0.097
		P (2-tailed)	0.285	0.753
		N	14	13
	PC3	Correlation Coefficient	0.169	0.050
		P (2-tailed)	0.564	0.871
		N	14	13

Table 5.7 Spearman´s rank correlations of mean principal component scores describing responses to auditory stimuli (calculated from 3 trials per individual), and the strength of the response to mother/offspring calls from Own and Other individuals.

			Own animal scores	**Other animal scores**
Responses to auditory stimuli	**PC1**	Correlation Coefficient	-0.387	0.140
		P (2-tailed)	0.154	0.633
		N	15	14
	PC2	Correlation Coefficient	-0.082	0.197
		P (2-tailed)	0.772	0.499
		N	15	14

There were also no significant differences in the mean PCs describing behavioural responses to novel object presentations and playbacks of auditory stimuli between responders and non-responders in the mother/offspring recognition experiment (Chapter 3), regardless of whether Own (Table 5.8) or Other (Table 5.9) individuals were considered.

Table 5.8 T-tests of the differences in mean principal component scores describing responses to novel object presentations (calculated from 3 trials per individual) and playbacks of auditory stimuli (calculated from responses pooled across up to 27 trials per individual), between **responders** and **non-responders** among **Other** (non-Own) individuals in the mother/offspring recognition experiment (for details see Chapter 3). Responders were defined according to whether they showed ear movements or looked towards the loudspeaker in response to playbacks of conspecific calls.

		T	df	P. (2-tailed)
Responses to novel object presentations	**PC1**	-0.536	11	0.603
	PC2	0.869	11	0.403
	PC3	0.274	11	0.789
Responses to auditory stimuli	**PC1**	0.472	12	0.645
	PC2	0.978	12	0.347

Table 5.9 T-tests of the differences in mean principle component scores describing responses to novel object presentations (calculated from 3 trials per individual), and to auditory stimuli (heterospecific playbacks; calculated from responses pooled across up to 27 trials) between **responders** and **non-responders** among **Own** individuals) in the mother/offspring recognition experiment (Chapter 3). Responders were defined according to whether they directed their body towards the loudspeaker or stood up in response to playbacks of conspecific calls.

		T	**df**	**P (2-tailed)**
Responses to novel object presentations	**PC1**	0.693	12	0.502
	PC2	-0.927	12	0.372
	PC3	0.011	12	0.992
Responses to auditory stimuli	**PC1**	-0.977	13	0.347
	PC2	-0.181	13	0.859

Finally, there was no significant correlation between the way that animals responded behaviourally to novel object presentations (Chapter 4, Table 4.3) and the way that they responded to auditory stimuli (heterospecific playbacks; Chapter 4, Table 4.4) (Table 5.10).

Table 5.10 Pearson's correlations between the mean PC scores describing the behavioural responses of individuals to the novel objects presentations (calculated from responses pooled across 3 trials per individual), and the PC scores describing their responses to auditory stimuli (heterospecific playbacks; calculated from responses pooled across up to 27 trials).

		Responses to novel objects		
		PC1	**PC2**	**PC3**
Responses to auditory stimuli				
PC1	Pearson Correlation	-0.015	0.056	-0.193
	P (2-tailed)	0.932	0.742	0.253
	N	37	37	37
PC2	Pearson Correlation	-0.198	0.283	0.163
	P (2-tailed)	0.241	0.089	0.337
	N	37	37	37

5.5 Discussion

I tested for correlations among the acoustic characteristics of cattle (mother-offspring contact calls), their ability to identify their own mother/offspring, and their behavioural responses in experiments designed to characterise their temperament, in which they were presented with novel objects) and auditory stimuli (playbacks of heterospecific vocalisations) in unconstrained circumstances in the field. Although, there was limited evidence of link between the acoustic characteristics of cattle vocalizations and their behavioural responses when new stimuli were presented, I found a positive correlation between Df (min) of cow LFCs, and possible indicators of boldness in the behavioural responses to both novel objects and auditory stimuli. Specifically, cows with higher Df (min) were more likely to sniff a novel object, and to call when they encountered it, and they were less likely to move away from the loudspeaker when they heard playbacks of heterospecific vocalisations. Filter characteristics, in particular the frequency spacing between successive formants (Df), provide the most reliable cues to body size (Fitch, 1997; Reby & McComb 2003). Therefore, the correlation implies that bigger cows gave bolder behavioural responses in these particular contexts. Sniffing, as mentioned before (see Section 4.3.3), is a behavioural response preceded always first by looking and approaching the object, which implies a degree of willingness to explore. Similarly, not moving away from the source of auditory stimuli would seem to be a highly plausible indicator of fearlessness. It is less clear, however whether calling in the novel object experiment, where cattle

were facing the challenge to explore a novel object but were not isolated or out of their normal environment context, should be interpreted as a bold or shy behaviour. Nevertheless, the presence of associations between df(min) and potentially bold behaviours in the two very different experimental contexts, and the marginally non-significant positive correlation between the second principal components describing responses to each of the novel object and auditory stimuli experiments (Table 5.3 and 5.4, respectively), is broadly consistent with the widely held view that a bold-shy syndrome commonly generates consistent differences in individual responses to stimuli across a wide range of contexts, including during social interactions, in threatening situations, during foraging contexts, whilst mating (Sih 2004; Reale et al.2007; Smith & Blumstein 2008, Biro & Stamps 2008; Sih et al. 2012).

The findings discussed above also imply that vocalisations encode information about the temperament of the caller. This is a relatively novel finding, although some other students on birds and other taxa have found similar relationships (Naguib et al. 2010; Guillette & Sturdy 2011), and it has potentially important implications for both pure and applied studies of animal behaviour. Despite the wealth of research into animal personality/temperament in recent years (Sih 2004; Reale et al.2007; Smith & Blumstein 2008, Biro & Stamps 2008; Sih et al. 2012), the possibility that individuals might signal the nature of their personalities to conspecifics has received little attention. If future work shows that my results

are typical for cattle and other species, this would imply that the ability to signal personality may be adaptive, which in turn would raise interesting questions about the costs and benefits not just of individual behavioural tendencies themselves, but also of the acquisition of knowledge about those tendencies in others. From an applied perspective, confirmation of my results would raise the question of whether it might be possible to assay temperament by analysing individual vocalisations, which of course would have great potential in studies of animal welfare and agricultural productivity. I have very limited ability to generalise from my results as they stand, but my study serves to highlight the possibilities: it is clear that we need to investigate the relationships between acoustic (and other) signals, and animal temperament/personality further.

Notwithstanding the weak evidence for a bold-shy continuum discussed above, the general impression gained from the analysis in this chapter is that the influence of consistency in individual behaviour across contexts was minimal. This could be explained by the importance of behavioural plasticity in determining responses to stimuli (Coleman & Wilson 1998; Neff & Sherman 2002; Dall et al. 2004; Harvey & Freeberg 2007).Behavioural plasticity allows individuals to adjust their behaviour over time and across contexts, to account for changes in the environment (Coleman & Wilson 1998; Neff & Sherman 2002; Dall et al. 2004; Harvey & Freeberg 2007). Plasticity is particularly likely to be observed in responses to changes in social environments (Coleman & Wilson 1998; Harvey &

Freeberg 2007), and the social environment was very different in the different experiments discussed in this thesis: animals encountered the novel objects individually, but they encountered heterospecific playbacks as a group, and they had their vocalisations and responses to vocalisations recorded in the context of the mother-offspring dyad. Thus, it is perhaps unsurprising that individual consistency was seemingly relatively unimportant when tested for across data-sets in this chapter.

In Chapter 4, whilst I found individual consistency within a single context when animals were repeatedly exposed to auditory stimuli, I failed for find such consistency in the behavioural response of cattle when they were presented with three different novel objects. Thus, consistent individual differences were found just in one of the contexts, and the general failure to find strong correlations in individual responses across contexts could reflect the fact that individuality was not important in determining reactions to novel objects. In general, these results suggest that shyness and boldness are context-specific, and may not exist as a one-dimensional behavioural continuum even within single context (Coleman & Wilson 1998). Nevertheless, few attempts have been made to quantify levels of variation in individual plasticity in the context of behaviour specifically (Dingemanse et al. 2010) and future work should be done in order to understand how individual consistency and plasticity interact. In order to do so, it will be

crucial to perform studies using experimental designs testing individual responses repeatedly within single contexts, and also in a variety of different contexts.

Interestingly, my results also showed a negative correlation between F0 in calf calls and PC1 from the behavioural response to the auditory stimuli, which correlated strongly with the boldest behaviours. Thus, calves with lower F0 tended to show the boldest response in the auditory stimuli experiment. F0 is a source component and its characteristics are due to the variation in sub-glottal pressure, and the length and shape of the vocal folds and their stress and tension (McComb & Reby 2005). F0 varies among individuals, and its range, variation and quality are likely to convey information on motivational state (McComb & Reby 2009). It could be assumed that contact calls, where mother and offspring have been out of sight for a long period of time, might be produced with a certain degree of arousal due to the urgency to find each other (especially in loud calls such cow HFCs and calf calls). Changes in respiration rate, tension of respiratory muscles, and salivation might occur under such conditions, which would lead to changes in the vocal apparatus and therefore in the acoustical parameters produced (Scherer 2003; Zei Pollermann & Archinard 2002). If variation was present among individuals in the degree of anxiety/urgency, and some of this variation was attributable to aspects of personality which were also reflected in responses to the auditory stimuli, this could explain the observed correlation.

Additionally, the fact that this correlation was found just for calf calls, and not for cow HFCs, could be explained if emotional state varied more in calves, which would have variable levels of experience in the field and hunger, than in their mothers.

Surprisingly, although several studies have investigated the acoustic structure of mother-offspring contact vocalizations, and established whether individual recognition using these vocalisations exist (e.g. Mexican free tail bats: *Tadarida brasiliensis mexicana*, Balcombe 1990; Australian fur seal: *Arctocephalus tropicalis,* Charrier et al. 2002; sheep: *Ovis aries*, Searby and Jouventin 2003; fallow deer: *Dama dama*, Torriani et al. 2006; walrus: *Odobenus rosmasus rosmasus,* Charrier et al. 2010; Australian sea lion: *Neophoca cinerea,* Pitcher et al. 2010; goats: *Capra hircus,* Briefer & McElligott 2011), at present, very little has been done to investigate the variation among individuals in the propensity to respond to close relatives which is evident in many of these studies. One possibility is that such variation reflects in some way the acoustic properties of contact vocalizations; perhaps some types of call are more easily recognised, and/or perhaps the acoustic properties of the calls of individuals reflect aspects of their personality, physiology or developmental state which in turn impact on their propensity to respond to maternal or offspring calls. Here, I made a tentative first attempt to look for such links. However, no correlations were found between the acoustic characteristics (F0 and Df(min)) of cattle vocalisations and the behavioural responses in the mother-offspring recognition

experiment. This result suggests that there is no relationship between the acoustic characteristics of individuals and their ability to identify their own mother or offspring in cattle. Furthermore, no link between the behavioural responses given by cattle during individual recognition playbacks and the PC scores generated from the behavioural responses given during exposure to novel objects and auditory stimuli was evident. This suggests behavioural responses towards conspecific vocalizations do not reflect any aspects of biological individuality which are also reflected in responses to situations which present possibilities for exploration (novel objects) or potential rewards/threats (heterospecific vocalisations).

An important caveat when discussing the results presented in this chapter is that my characterisation of cattle behaviour, and the subsequent analysis of that behaviour, has limited power to answer the questions posed. The principal components extracted from data collected in the novel object and auditory stimuli experiments, and the behaviours which they summarise, are rather crude measures of what were seemingly quite complex responses in the field. Furthermore, the small sample sizes available in most analyses (typically < 10) limit my ability to generalise from these results owing to a mis-match between the identities of the animals whose vocalisations were recorded, and those which took part each of the experiments. Finally, there is a high probability that those "significant" results which were identified reflect type-1 erorrs: I conducted a

total of 64 statistical tests in this chapter, of which only three were significant. The probability of obtaining this number of significant results (or more) by chance, if all of the null hypotheses were in fact true, is 40 %. Thus, in general, caution should be exercised when interpreting these results, and clearly further experiments are required to test fully the hypotheses that there will be relationships between the ability or propensity of individuals to recognise close relatives, the characteristics of those individuals' vocalisations, and their response to novel or threatening situations. Nevertheless, the results presented here provide tantalising hints about the potential importance of some of these relationships, some of which have not previously been explored in studies of this kind.

5.6 References

Balcombe, J.P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana.* Animal Behaviour 39, 960-966**.**

Burrow, H.M. 1997. Measurements of temperament and their relationship with performance traits of beef cattle. Animal Breeding Abstracts 65, 477-495.

Biro, P.A. & Stamps, J.A. 2008. Are animal personality traits linked to life-history productivity? Trends in Ecology and Evolution 23, 361–368.

Boissy, A., & Bouissou, M.F. 1995. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Applied Animal Behaviour Science 46, 17-31.

Briefer, E., McElligott, A. G. 2011. Mutual mother-offspring vocal recognition in an ungulate hider species (*Capra hircus*). Animal Cognition 14, 585-598.

Briefer, E. F. 2012. Vocal expression of emotions in mammals: mechanisms of production and evidence. Journal of Zoology 288, 1-20.

Carter, A.J. Heinsohn, R. Goldizen, A.W., & Biro, P.A. 2012. Boldness, trappability and sampling bias in wild lizards. Animal Behaviour 83, 1081-1058.

Charrier, I., Mathevon, N. & Jouventin, P. 2002. How does a fur seal mother recognize the voice of their pup? An experimental study of *Arctocephalus tropicalis*. Journal of Experimental Biology 205, 603-622.

Charrier, I., Aubin, T., & Mathevon. 2010. Mother-calf vocal communication in Atlantic walrus: a first field experimental study. Animal cognition 13, 471-482.

Coleman, K. &, Wilson, D.S. 1998. Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. Animal Behaviour 56, 927–936.

Dall, S. R. X., Houston, A. I. & McNamara, J. M. 2004 The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecology Letters 7, 734–739.

Decker, R.A, & Griffen, B.D. 2012. Correlating context-specific boldness and physiological condition of female sand fiddler crabs (*Uca pugilator*). Journal of Ethology 30, 403-412.

Dingemanse, N. J., Kazem, A. J. N., Re´ale, D. & Wright, J. 2010 Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology and Evolution 25, 81–89.

Fitch, W. T. 1997. Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. Journal of the Acoustical Society of America 102, 1213-1222.

Forkman, B. ,Boissy, A., Meunier-Salaun, M.C., Canali, E., & Jones, R.B. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horse. Physiology & Behaviour 92, 340-374.

Gibbons, J., Lawrence, A., & Haskell, M. 2009. Responsiveness of dairy cows to human approach and novel stimuli. Applied Animal Behaviour Science 116, 163-173.

Guillette, L.M., & Sturdy, C. B. 2011. Individual differences and repeatability in vocal production: stress induced calling exposes a songbird's personality. Naturwissenschaften 98, 977-981.

Harcourt, J.L., Sweetman, G., Johnstone, R.A., & Manica A. 2009. Personality counts: the effect of boldness on shoal choice in three-spined sticklebacks. Animal Behaviour 77, 1501-1505.

Harvey, E. M., & Freeberg T. M. 2007. Behavioural consistency in a changed social context in carolina chickadees. The Journal of General Psychology 134, 229-245.

Herskin, M. S., Kristensen, A.M., Munksgaard, L. 2004. Behavioural responses of dairy cattle toward novel stimuli presented in the home environment. Applied Animal Behaviour Science 89, 27-40.

IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. IBM Corp. Armonk, NY, USA.

Kilgour, R.J., Melville, G.J., & Greenwood, P.L. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans and novelty. Applied Animal Behaviour Science 99, 21-40.

Kluever, B.M., Howery, L.D., Breck, S.W., & Bergman, D.L. 2009. Predator and heterospecific stimuli alter behavior in cattle. Behavioral Processes 81, 85-91.

Kralj-Fiser, S., Weiß, B. M., & Kotrschal, K. 2010. Behavioural and physiological correlates of personality in greylag geese (*Anser anser*). Journal of Ethology 28, 363-370.

Laporte, I., Muhly, T.B., Pitt, J.A., Alexander, M., & Musiani, M. 2010. Effects of wolves on elk and cattle behaviours: implications for livestock production and wolf conservation. PLos One. 5, e11954.

Leavesley, A. & Magrath, R. D. 2005. Communicating about danger: urgency alarm calling in a bird. Animal Behaviour 70, 365–373.

Macedonia, J. M. & Evans, C. S. 1993. Variation among mammalian alarm call systems and the problem of meaning in animal signals. Ethology 93, 177–197.

Manser, M. B. 2001. The acoustic structure of suricates'alarm calls varies with predator type and the level of response urgency. Proceedings of the Royal Society B 268, 2315–2324.

McComb, K, & Reby, D. 2005. Vocal communication networks in large terrestrial mammals. In: McGregor, P. K (Ed.) Animal communication Networks. Cambridge Press. pp. 372-389.

McComb, K., & Reby, D. 2009. Communication in terrestrial mammals. In: Squire, L. R. (Ed.) Encyclpedia of Neuroscience, vol. 2. Oxford Academic Press. pp. 1167-1171.

Naguib, M., Kazek, A., Schaper, S.V., Oers, K., & Visser, M.E. 2010. Singing activity reveals personality traits in great tits. Ethology 116, 763-769.

Neff, B. D. & Sherman P.W. 2002. Decision making and recognition mechanisms. Proceedings of the Royal Society of London B 269, 1435–1441.

Pitcher, B.J., Harcourt R.G., & Charrier, I. 2010. Rapid onset of maternal vocal recognition in a colonially breeding mammal, the Australian sea lion. PLoS ONE 5**,** 1-7.

Rèale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews 82, 291-318.

Reby, D., & McComb, K. 2003. Anatomical constrains generate honesty: acoustic cues to age and weight in the roars of red deer stags. Animal Behaviour 65, 519-530.

Scherer, K. R. 2003. Vocal communication of emotion: A review of research paradigms. Speech Communication 40, 227-256.

Searby, A., & Jouventin, P. 2003. Mother-lamb acoustic recognition in sheep: a frequency coding. Proceeding of the Royal Society B 270, 1765-1771.

Sih, A. 2004. Behavioural syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution 19, 372-378.

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. 2012. Ecological implications of behavioural syndromes. Ecology Letters 15, 278-289.

Smith, B.R. & Blumstein, D.T. 2008. Fitness consequences of personality: a meta-analysis. Behavioural Ecology 19, 448–455.

Torriani, M. V. G., Vannoni, E. & McElligott, A. G. 2006. Mother-young recognition in an ungulate hider species: a unidirectional process. American Naturalist 168, 412-420.

Van D Kooij, Kuijpers, A.H., Schrama, J.W., van Eerdenburg, F.J.C.M., Schouten, W.G.P., & Tielen, M.J.M. 2002. Can we predict behaviour in pigs?: Searching for consistency in behaviour over time and across situations. Applied Animal Behaviour Science 4, 293-305.

Welp, T., Rushen, J., Kramer, D.L., Festa-Bianchet, M., de Pasitlle, A.M.B. 2004. Vigilance as a measure of fear in dairy cattle. Applied Animal Behaviour Science 87, 1-13.

Zei Pollermann, B. & Archinard, M. 2002. Acoustic patterns of emotions. In: Keller, E., Bailly, G., Monaghan, A., Terken, J. & Huckvale, M. (Eds). Improvements in speech synthesis. Chichester, John Wiley & Sons. pp. 237–245pp.

CHAPTER SIX

General discussion

6. General discussion

My research contributes to our relatively meagre knowledge about the behaviour of domestic cattle, but also has wider significance. Specifically, the results described in my thesis provide evidence for individuality in animal communication and in some aspects of temperament and bidirectional mother-offspring recognition, and includes a preliminary exploration of possible associations between these phenomena. Additionally, the results provide insights into cattle behaviour which are potentially directly applicable in the field of animal welfare.

Although I have focused my research on a domesticated species, which provide highly tractable experimental models, I have emphasised throughout the thesis the importance of the fact that the conditions in which my experiments were conducted were designed to mimic as closely as possible the natural environment in which wild bovids live. Despite the existence of some feral cattle populations, which could provide basic insights into the social structure of the ancestral species, very little information about behaviour has been gleaned from these populations. Comparisons with other wild bovid species can tell us something more about the adaptive value of behaviour in modern cattle, but the fact that no cattle exist today that have not been domesticated makes the process of

understanding the origins and function of cattle behaviour a difficult one. In order to answer basic evolutionary and ecological questions about cattle behaviour, I have thus highlighted the importance of studying a cattle population living under relatively natural conditions.

In Chapter Two I investigated the acoustic characteristics of contact calls in cows and calves and determined that they are individually distinctive. To my knowledge, this is the first study assessing vocalizations in cattle from the source-filter theory perspective (Fant 1960; Titze 1994) and it provides a baseline description of the acoustic properties of cattle contact calls upon which future studies can build. Furthermore, the results of this study strongly support the idea that in cattle, as has been found in other mammal species, the source characteristics do not reflect an individual's age or body size, and in contrast that filter-related characteristics provide more reliable indicators of age/body size (Reby & McComb 2003; McComb & Reby 2005).

Although my thesis provides the first full acoustic description of vocalizations in cattle, I considered only mother-offspring contact calls. In order to be able to use vocalizations as indicators of animal welfare (Grandin 1998; Watts & Stookey 1999 & 2000; Manteuffel et al. 2004; Ikeda & Ishii 2008), future studies providing

the acoustic characteristics of the full range of cattle vocalizations from the source-filter theory point of view need to be done.

Cattle vocalizations have been proposed by many researchers as potential indicators of animal welfare (Grandin 1998; Watts & Stookey 1999 & 2000; Manteuffel et al. 2004; Ikeda & Ishii 2008). However, very few studies have investigated this idea. Furthermore, what little research there is into cattle vocalizations has been done almost entirely in highly stressful situations for the animals, or under intensive husbandry conditions. These are conditions in which it seems extremely unlikely that "normal" behaviours will be observed (Miranda-de la Lama & Mattiello 2010). Mine is the first study to assess mother-offspring vocalisations made by cattle where no artificial isolation or invasive human intervention was used. Therefore, I consider that my results have the potential to serve as a reference point for future studies of the significance of the characteristics of vocalisations for animal welfare.

In addition to the implications for animal welfare research, my results have ecological and evolutionary relevance, helping us to understand more fully, for example, the nature and adaptive significance of maternal and offspring behaviour. The experiment described in Chapter Three confirmed for the first time that the acoustic individuality observed in cow and calf vocalizations

facilitates bidirectional individual recognition between mothers and their offspring Vocal parameters with enough individuality can potentially be used as "signatures" for individual recognition, and these signatures can greatly facilitate social interactions between animals where individual specific relationships (evidenced by distinctive behavioural responses to different conspecifics) are important (Shapiro 2009). The ability to recognize other individuals offers an adaptive advantage in situations such as parents searching for their offspring (Chapter Three), which is particularly important in gregarious species such as bovids. The fact that calves display following behaviour relatively soon after birth, and the socially mingle with other conspecifics, might mean that selection has favoured individual vocalizations. It is not possible, however, to discard the possibility that cattle could have developed mother-offspring bidirectional recognition as an adaptation to the farm environment after 10, 000 or 8000 years under domestication (Bailey et al. 1996; Bradley & Magee 2006). Likewise, this history of intensive husbandry opens another possible explanation, where bidirectional recognition could have been an acquired (learnt) ability in cattle, since they tend to be captive in small areas and therefore mother and offspring spend more time together.

The main objective of research in animal temperament is to conceptualize, from an evolutionary perspective, the very general phenomenon that individuals from the same population differ markedly and consistently in the behaviours they

exhibit (Sih et al. 2003; Bell 2007; Réale et al. 2007; Harvey & Freeberg 2007; Stamps & Groothuis 2010). With this concept as a point of reference, in Chapter Four, I looked for evidence of consistent temperament in cattle in two widely different contexts: in response to visual stimuli (novel objects), and in response to acoustic stimuli (heterospecific playbacks). The results obtained showed limited evidence of individuality within contexts; cattle showed individual consistency in response to acoustic stimuli and therefore could be assigned behavioural types (bold or shy) , as in previous studies performed in cattle (Van Reenen et al. 2004; Kligour et al. 2006; Jones & Godin 2010; Kurvers et al. 2010). However, although cows and calves behaved consistently differently in response to encounters with novel objects, no additional individuality was detected, and in Chapter Five it was seen that there was no strong evidence of individual behavioural consistency across the two different contexts.

There is ample evidence that individuals can adjust their behaviour in response to changing environmental conditions (behavioural plasticity; Mathot et al. 2012), including changes in the social environment (Coleman & Wilson 1998; Harvey & Freeberg 2007). This behavioural plasticity has the potential to interact with, or interrupt, the impact of temperament on behavioural consistency. Thus, if social or other environmental context strongly affects behavioural responses, then researchers cannot necessarily predict an individual's behaviour when its context changes (Harvey & Freeberg 2007). The lack of individual behavioural consistency

seen across contexts in Chapter Four is therefore perhaps not hugely surprising, given that the novel object and auditory stimuli experiments presented animals with markedly different environmental contexts. Importantly, analysis in Chapter Four suggested that individual responses to auditory stimuli, which unlike the responses to novel objects were recorded in a social context, were strongly influenced by the behaviour of the rest of the herd. Perhaps these differences in environmental context between the two experiments generated sufficient plasticity in responses to mask any underlying individual behavioural consistency across contexts, such as that expected to result from the existence of a bold-shy behavioural syndrome. Attempts to measure the importance of temperament across contexts is potentially further complicated by variation in plasticity itself, both within individuals among environments (Dingemanse et al. 2010), and among individuals/genotypes across contexts (Brommer et al. 2005; Nussey et al. 2005; Nussey, et al. 2007). The job of fully disentangling plasticity and consistency in behaviour, that most labile of traits, will require much work in the future.

Attempting to establish possible correlations among all the traits measured in experiments carried out in this thesis, Chapter Five showed limited evidence of association between the acoustic characteristics of cattle contact calls and responses to novel objects and auditory stimuli. Interestingly the associations were found in calf calls and in cow LFCs, but not cow HFCs. The parameter that positively correlated for cow LFCs was the minimum formant spacing (Df (min))

while a negative correlation for calf calls was found in their fundamental frequency (F0). Interestingly, our findings in Chapter Two demonstrated that cow LFCs are the vocalizations with greater individuality. This fact, combined with the correlations with behaviour seen in Chapter Five, implies that LFCs are for some reason better predictors of individual identity and behaviour than HFCs. We can only speculate about why this might be. LFCs were exclusively produced relatively early in calf development, at a time when new-born calves by definition are naïve about differences among individual adults, and must find a way of identifying their mothers. Later in development, when HFCs were the only type of call observed, calves may already be very sensitive to subtle differences among the calls of the adults in their environment, and may also have learnt to use other cues to identify their mothers.

The positive correlation in calves between vocalisation F0 and responses to the auditory stimuli suggest that the level of arousal experienced by calves in in response to heterospecific calls, which could be indicative of potential rewards (e.g. food from a familiar human) or threats (e.g. from unfamiliar dogs or wolves), could be codified within their contact calls. Since F0 might convey information on motivational state (McComb & Reby 2009), more research linking emotions with source-filter parameters in vocalizations needs to be done in the future. My results were an attempt to establish an association between acoustic characteristics of contact calls and temperament. Nevertheless, very little is

known at the moment about vocalizations and the relationship with emotions: it is plausible that the acoustic properties of calls could encode information about activation level (aroused vs. calm) and/or hedonic valence (pleasant/positive vs. unpleasant/negative), but this has never been investigated properly in mammals (Zei Pollermann & Archinard 2002; see review Briefer 2012).

Very few studies have related acoustic characteristics and temperament (but see Naguib et al. 2010; Guillette & Sturdy 2011), and no research of this sort has been done in domestic animals, or in the context of mother-offspring vocalizations. This is especially surprising considering that, in domestic species such as cattle, it is feasible to measure accurately both individual acoustic characteristics and to perform appropriate temperament experiments. Furthermore, the link between personality expressed outside the family context, and behaviours expressed during parent–offspring and sibling–sibling interactions has rarely been considered from an evolutionary viewpoint (but see Sih & Bell 2008; Roulin et al 2010). Although, my results attempted to correlate acoustic parameters with behavioural responses in mother-offspring recognition trials, no strong patterns were evident. However, the statistical power of my study was low, and it is entirely possible that a link should exist, but we have no other equivalent research to call upon in evaluating the validity of the hypothesis being tested. Therefore, further experiments are clearly necessary, in cattle and other species, if we are to establish whether the fact that some individuals respond to the calls

of their close kin (mothers/offspring), whilst others do not, is in some way indicative of more general differences in temperament/personality among individuals.

Similarly, my results did not show evidence of correlation between mother-offspring behavioural response and individual temperament in different contexts. Nevertheless, it has been argued that personality traits are expected to be functionally related to specialized behaviours expressed in the family, and thereby have the potential to influence (and be influenced by) the evolution and resolution of family conflicts (Roulin et al. 2010). There is a possibility that parental responsiveness to offspring signals may be associated with temperament/personality traits. For example, in great tits, parents who quickly and superficially explore a novel environment take more risk to protect their offspring than parents who explore the same environment slowly but thoroughly (Hollander et al. 2008). Of particular importance then is to determine whether parent and offspring personality determines parental effort towards the offspring (e.g. food supply), and whether family interactions influence the ontogeny of personality (Roulin et al. 2010).

Finally, given that my experiments were conducted in relatively uncontrolled, open-field settings, and had relatively low statistical power, the detection of at least some individuality in behaviour suggests that temperament may be

important not just in the confines of the cattle yard, where farmers become familiar with particularly “recalcitrant”, “friendly” or “nervous” individuals, but more generally in the lives of domestic cattle and quite probably in the lives of their ancestors. Considering that in domestic animals, the opportunity to express normal social behaviours is usually limited by captivity and husbandry systems (Miranda-de la Lama & Mattiello 2010), I strongly suggest that future research into cattle behaviour tests animals under the most natural conditions possible. In that way, behavioural responses can be observed which reflect accurately the recent and ancient evolutionary history of the species. While some have questioned the usefulness of data collected from animals reared and observed in captivity in attempts to understand the adaptive significance of behaviour (Miranda-de la Lama & Mattiello 2010; Zeder 2012; Overveld & Matthysen, 2013), there is good evidence that captive-reared animals are capable of displaying the full range of behaviours observed in their wild counterparts (Price 1984; Meager et al. 2011). For example, studies in fish have demonstrated that mechano-acoustic stimuli can invoke a range of responses that include both fast-start escapes and behaviours that are usually associated with chemical and visual predator cues, such as freezing and reducing activity in the risky habitat (Kelley, 2008). Although it was predicted that wild fish would display a broader behavioural repertoire than the hatchery-reared fish (fish that live in facilities where eggs are hatched under artificial conditions), the full range of responses was displayed by both fish types (Meager et al. 2011). Examples such as this

suggest that observations of cattle behaviour under the most natural conditions possible will allow a more comprehensive understanding of the extent to which modern intense husbandry modifies behaviour and perhaps compromises welfare.

5.1 References

Bailey, J. F., M. B. Richards, V. A. Macaulay, I. B. Colson, I. T. James, D. G. Bradley, R. E. Hedges, & Sykes, B. C. 1996. Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species. Proceedings of the Royal Society of London B Biological Sciences 263: 1467–1473.

Bell, A. M. 2007. Future directions in behavioural syndromes research. Proceedings of the Royal Society B 274, 755-761.

Bradley, D.G., & Magee, D.A. 2006. Genetics and the origins of domestic cattle. In: Zeder, M.A., Bradley, D.G., Emshwiller, E., & Smith, B.D. (Eds.) Documenting domestication: new genetic and archaeological paradigms. London: University of California Press. pp 317–328.

Briefer, E. F. 2012. Vocal expression of emotions in mammals: mechanisms of production and evidence. Journal of Zoology 288, 1-20.

Brommer, J.E., Merila, J., Sheldon, B.C., & Gustafsson, L. 2005. Natural selection and genetic variation for reproductive reaction norms in a wild bird population. Evolution 59, 1362–1371.

Coleman, K. &, Wilson, D.S. 1998. Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. Animal Behaviour 56, 927–936.

Dingamanse, N.J. Kazem, A.J.N., Réale, D., & Wright, J. 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology and Evolution 25, 81-89.

Fant, G. 1960. Acoustic theory of speech production. Mouton, The Hague.

Grandin, T. 1998. The feasibility of using vocalization scoring as an indicator of poor welfare during cattle slaughter. Applied Animal Behaviour Science 56, 121-128.

Guillette, L.M., & Sturdy, C. B. 2011. Individual differences and repeatability in vocal production: stress induced calling exposes a songbird's personality. Naturwissenschaften 98, 977-981.

Harvey, E. M., & Freeberg T. M. 2007. Behavioural consistency in a changed social context in carolina chickadees. The Journal of General Psychology 134, 229-245.

Ikeda, Y., & Ishii, Y. 2008. Recognition of two psychological conditions of a single cow by her voice. Computers and Electronics in Agriculture 62, 67-72.

Jones, K.A., & Godin, J.G.J. 2010. Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. Proceedings of the Royal Society B. 277, 625-62.

Kelley, J. L. 2008. Assessment of predation risk by prey fishes. In: Magnhagen, C., Braithwaite, V., Forsgren, E. & Kapoor, N. N. (Eds.) Fish Behaviour. Enfield, NH: Science Publishers .pp. 269–302.

Kilgour, R.J., Melville, G.J., & Greenwood, P.L. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans and novelty. Applied Animal Behaviour Science 99, 21-40.

Kurvers, R.H.J.M., van Oers, K., Nolet, B.A., Jonker, R.M., van Wieren, E., Prins, H.H.T., & Ydenberg, R.C. 2010. Personality predicts the use of social information. Ecology Letters 13, 829-837.

Manteuffel, G., Puppe, B., & Schon, P. 2004. Vocalizations of farm animals as a measure of welfare. Applied Animal Behaviour Science 88, 163-182.

Mathot, K.J., Wright, J., Kempaenaers, B., & Dingamanse, N. J. 2012. Adaptative strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. Oikos 121, 1009-1020.

Meager, J.J., Rodewald, P., Domenici, P. Fernö, A., Järvi, T., Skjæraasen, J. E., & Sverdrup, G.K. 2011. Behavioural responses of hatchery-reared and wild cod *Gadus morhua* to mechano-acoustic predator signals. Journal of Fish Biology 78, 1437–1450.

McComb, K, & Reby, D. 2005. Vocal communication networks in large terrestrial mammals. In: McGregor, P. K (Ed.) Animal communication Networks. Cambridge Press. pp. 372-389.

McComb, K., & Reby, D. 2009. Communication in terrestrial mammals. In: Squire, L. R. (Ed.) Encyclopedia of Neuroscience, vol. 2. Oxford Academic Press. pp. 1167-1171.

Miranda-de la Lama, G.C., & Mattiello, S. 2010. The importance of social behaviour for goat welfare in livestock farming. Small Ruminant Research 90, 1-10.

Naguib, M., Kazek, A., Schaper, S.V., Oers, K., & Visser, M.E. 2010. Singing activity reveals personality traits in great tits. Ethology 116, 763-769.

Nussey, D.H., Wilson, A.J., & Brommer, J.E. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. Journal of Evolutionary Biology 20, 831–844.

Nussey, D.H., Postma, E., Gienapp, P., & Visser, M.E. 2005. Selection on heritable phenotypic plasticity in a wild bird population. Science 310, 304–306.

Overveld van, T., & Matthysen, E. 2013. Personality and Information Gathering in Free-Ranging Great Tits. Plos One 8, e49645.

Price, E. O. 1984. Behavioural aspects of animal domestication. Quarterly Review of Biology 59, 419-422.

Rèale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews 82, 291-318.

Reby, D., & McComb, K. 2003. Anatomical constrains generate honesty: acoustic cues to age and weight in the roars of red deer stags. Animal Behaviour 65, 519-530.

Roulin, A., Dreiss, A., & Kölliker, M. 2010. Evolutionary perspective on the interplay between family life and parent and offspring personality. Ethology. 116, 787-796.

Shapiro, A. D. 2009. Recognition of individuals within the social group: signature vocalizations. In Brudzynky S. M. (Ed.) Handbook of mammalian vocalizations-an integrative neuroscience approach. Academic press, London, pp 495-503.

Sih, A., & Bell, A. 2008. Insights from behavioural syndromes for behavioural ecology. Advances in the Study of Behaviour 38, 277-281.

Sih, A., Kats, L.B., & Maurer, E.F. 2003. Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. Anim. Behaviour 65, 29–44.

Stamps, J. A., & Groothuis, T.G.G. 2010. The development of animal personality: relevance, concepts and perspectives. Biological Reviews 85, 301-325.

Titze, I. 1994. Principles of voice production. Prentice Hall, Englewood Cliffs.

Van Reenen, C.G., Engel, B., Ruis-Heutinck, L.F.M., Van der Werf, J.T.N., Buist, W.G., & Blokhuis, H.J. 2004. Behavioural reactivity of heifer calves in potentially alarming test situations: a multivariate and correlational analysis. Applied Animal Behaviour Science 85, 11-30.

Watts, J. M., & Stookey, J. M. 1999. Effects of restraint and branding on rates and acoustic parameters of vocalization in beef cattle. Applied Animal Behavioural Science 62, 125-135.

Watts, J. M., & Stookey, J. M. 2000. Vocal behaviour in cattle: the animal's commentary on its biological processes of welfare. Applied Animal Behaviour Science 67, 15-33.

Zeder, M. A. 2012. Pathways to Animal Domestication. In: Biodiverity in Agriculture: Domestication, Evolution, and Sustainability. Gepts, P., Famula, T. R., Bettinger, R. L., Brush, S. B., Damania, A. B., McGuire, P. E., & Qualset, C. O. (Eds.) Cambridge University Press, UK. pp. 227-259.

Zei Pollermann, B. & Archinard, M. 2002. Acoustic patterns of emotions. In: Keller, E., Bailly, G., Monaghan, A., Terken, J. & Huckvale, M. (Eds). Improvements in speech synthesis. Chichester, John Wiley & Sons. pp. 237–245pp.